Molar Volume of Polystyrene in Solution

By Gisuke MESHITSUKA and Yoshiki MATSUI

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Introduction

Osmotic pressure measurements have been made on fractions of polystyrene in toluene, benzene and methylethylketone at 20°C, and the maximum molar volume b_{max} of solute have been calculated by the relation (1), which has already been reported by us[1],

$$A_2 = \frac{b_{max}}{M^2} \qquad (1)$$

where A_2 represents the second virial coefficient of osmotic pressure, M the molecular weight, and b_{max} is the volume occupied by the solute molecule at its highest degree of expansion.

Many attempts have been made to deduce the relation between the degree of expansion and the molecular weight of chain molecules in dilute solutions. For example Flory[2] has shown that special interferences between distantly connected segments of a chain lead to the relation

$$R \propto M^{\alpha}, \qquad (2)$$

where R is the average radius of the expansion of chain molecules in dilute solution, and the parameter α changes between 0.5 and 0.6 with varying solvent-polymer systems.

We have examined the relation between b_{max} and M, and obtained values of α for polystyrene in toluene, benzene and methylethylketone at 20°C.

Experimental

Preparation of Polymer Samples.—Styrene monomer was sealed in tubes in atmosphere. The tubes were placed in a thermostat at 120°C for nine and a half hrs. The contents were taken up in benzene and then monomer and benzene were removed from the mixtures by the method of vacuum sublimation.

The polystyrene was separated into 19 fractions by fractional precipitation, vaporizing benzene and butanol mixture solution[3]. These fractions were reprecipitated with CH_3OH from benzene solutions and finally dried by vacuum sublimation of benzene from benzene solutions frozen by dry ice.

Measurement of Osmotic Pressure.—Osmotic pressure measurements were made in Zimm and Meyerson type osmometers, kept in a thermostat regulated to 20.000±0.003°C. Denitrated collodion was used as membranes. A static method of measurement was employed. Equilibrium was attained in about 10 hrs. The values of the osmotic pressure were accepted only when it remained constant for 12 hrs.

Results and Discussion

The osmotic pressure measurements were made on six fractions in toluene, and three fractions in both benzene and methylethylketone. Some of the plots of π/c against c are shown in Fig. 1. The unit of concentration is g.×cm^{-3} and that of pressure is g.×cm^{-2} and hence π/c is in cm.

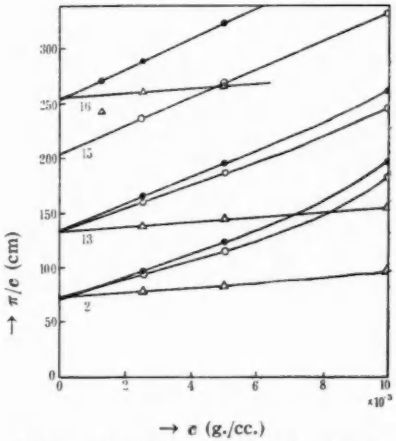

Fig. 1. Variation of π/c with c for polystyrene at 20°C; ○ toluene, ● benzene, △ methylethylketone.

Extrapolation to $c=0$ was made by free-hand drawing, and the molecular weight M was calculated from the intercept, and the second virial coefficient A_2 from the initial slope of the curve. The b_{max} was calculated by the relation (1) using the values of M and A_2. These results are summarized in Table I.

1) G. Meshitsuka, This Bulletin, **25**, 309 (1952).
2) P. J. Flory, *J. Chem. Phys.*, **17**, 303 (1949).
3) T. Titani, G. Meshitsuka, and Y. Matsui, *Chem. High Polym., Japan*, **10**, 175 (1953).

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TABLE I
THE MOLECULAR WEIGHTS M, THE SECOND VIRIAL COEFFICIENTS A_2 AND THE MAXIMUM MOLAR VOLUMES b_{max}

Fraction No.	$M \times 10^{-4}$	$A_2 \times 10^4$ (cc/g²)			$b_{max} \times 10^{-4}$ (l/mole)		
		Toluene	Benzene	Methyl-ethyl-ketone	Toluene	Benzene	Methyl-ethyl-ketone
2	33.6	3.25	4.25	0.80	3.7	5.4	0.93
5	27.2	3.95	—	—	2.9	—	—
11	21.8	4.28	—	—	2.0	—	—
13	18.7	4.28	5.02	0.94	1.5	1.8	0.33
15	12.2	4.98	—	—	0.74	—	—
16	9.8	—	5.62	1.07	—	0.54	0.10
17	6.0	6.02	—	—	0.22	—	—

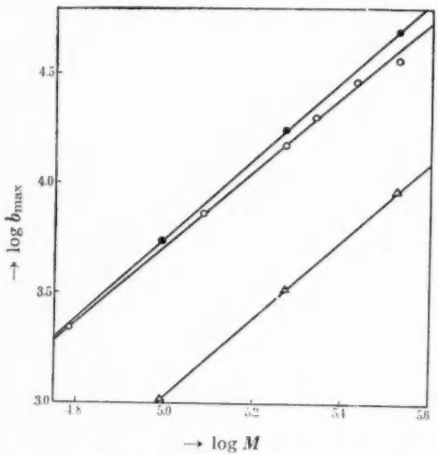

Fig. 2. Variation of log b_{max} with log M for polystyrene at 20°C; ○ toluene, ● benzene, △ methylethylketone.

Assuming the molecules to be sphere, b_{max} must be expressed as

$$b_{max} = 4/3\pi R^3 \qquad (3)$$

Inserting the equation (2), it follows that

$$b_{max} = KM^{3\alpha}, \qquad (4)$$

or

$$\log b_{max} = \log K + 3\alpha \log M, \qquad (5)$$

where K is a constant.

Fig. 2 is the plots of log b_{max} against log M for our results on the polystyrene in toluene, benzene and methylethylketone, which shows straight lines. The values of K and α determined are shown in Table II.

TABLE II
K AND α FOR POLYSTYRENE

Solvent	$K \times 10^5$	α
Toluene	1.46	0.57
Benzene	0.69	0.60
Methylethyl-ketone	0.17	0.59

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*On the Molecular Rotations of Polypeptides of Alanine**

By Hideo MURAKAMI

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Introduction

Abderhalden and Gohdes observed the specific rotations $[\alpha]_D$ for a series of polypeptides of *l*-alanine.[1] Levene and Yang also observed them for a series of *d*-alanine, and obtained almost equal data concerning their absolute values.[2]

* This work was reported at the meeting for general discussion of protein structure held on May 20, 1951, in Osaka, under the auspices of the Chemical Society of Japan.

1) E. Abderhalden and W. Gohdes, *Ber.*, **64**, 2070 (1931).
2) P. A. Levene and P. S. Yang, *J. Biol. Chem.*, **99**, 405 (1932).

As was pointed out by Kauzmann and Eyring, the molecular rotation per one alanine residue $[M]_D/n$ (n is the number of residues) increases remarkably as the polypeptide chain becomes longer. This fact indicates that the neighbouring alanine residues are not optically independent and there will be some interactions. Kauzmann and Eyring gave an interesting suggestion for this point based on their consideration for the optical rotations of sugar.[3] Firstly, they noticed that the molecular rotations of ring compounds such as arabinose, xylose, mannose, galactose, glucose, etc. are relatively great (30°~260°) compared with those of corresponding alcohols (less than 10°). They interpreted these differences between ring and chain compounds by considering the statistical free rotation (restricted rotation) around a single bond. That is, in case of open chain compounds, the above-mentioned statistical free rotation is allowed, and the first order effect of optical rotation is almost entirely diminished. On the other hand, in the case of ring compounds, the rotation around the single bond is prevented on account of their rigid structures and the first-order effect of optical rotation can contribute considerably. Taking these facts into account, they suggested that the molecules of polypeptides of alanine may also form a ring involving hydrogen bond or some other type of bond. Based on this suggestion, we try to explain theoretically the observed rotations in Table I by assuming the probable model for the configuration of polypeptide.

TABLE I

OBSERVED VALUES OF SPECIFIC AND MOLECULAR ROTATION

Polypeptides	$[\alpha]_D$ in 2 N HCl		$[M]_D$	$[M]_D/n$ n is the number of residues
	Abderhalden	Levene	Levene	
alanine	+ 14.5°	− 14.5°	− 13°	−13°
alanyl-alanine	− 37.6°	+ 36.5°	+ 58°	+29°
di-alanyl-alanine	− 77.3°	+ 77.5°	+179°	+60°
tri-alanyl-alanine	−115°	+115°	+347°	+87°
tetra-alanyl-alanine	−133°	+132.5°	+494°	+99°

I. MOLECULAR MODEL

Ring Strncture of Polypeptide Chain In order to calculate the molecular rotation theoretically, it is necessary that the detailed structure of the molecule is known. Conversely, if the calculated results for a probable model agree well with experimental results, the assumed model becomes more reliable.

The configurations of the polypeptide chain in protein have been studied extensively, and the numerous probable models for them have been presented by many authors.[4-9] These models are mostly based on the experimental data for crystal, fiber or film of highly polymerized large polypeptide molecules whether they be natural or synthetic.

At present, however, we are researching the configurations of small polypeptide molecules in dilute water solution, so the situations are considerably different from the above-described ones. Moreover, our model must be rigid enough and correspond to the ring structure which was demanded by Kauzmann and Eyring. Therefore, it is required that not only the known bond lengths and bond angles are satisfied approximately, but also many hydrogen bonds are formed to construct the rings. Further, it can be expected that the rigidity of the ring becomes greater as the ring becomes smaller, so long as the geometrical conditions for the stability of hydrogen bonds and internal rotations are satisfied.

Considering these conditions, we picked out (2_7 a) the model of Huggins and (2_7 b) the model of Shimanouchi and Mizushima as the most probable ones. In these models, sevenmembered rings are formed by hydrogen bonds, and concerning internal rotation, the stable configurations of the polypeptide chain are kept. At the present stage, however, the application of these models should be limited to the configurations of the relatively short polypeptide chain in solution. For the configurations of highly polymerized polypeptide chain in protein, some other factors should be taken into account, and their complete determination is a task for future study.

Equilibrium between Isomers Since the above-mentioned models have been presented for the folded α-type keratin, we denote the models (2_7 a) and (2_7 b) as $\alpha_{(1)}$ and $\alpha_{(2)}$ respectively. The only difference between $\alpha_{(1)}$ and $\alpha_{(2)}$ is the relative internal orientation between the side chain and the main chain, that is, in the case of $\alpha_{(1)}$, its orientation exists in G (Gaush) form, and in the case of

3) W. Kauzmann and H. Eyring, *J. Chem. Phys.*, **9**. 41 (1941).
4) M. L. Huggins, *Chem. Rev.*, **32**, 195 (1943).
5) T. Shimanouchi and S. Mizushima, *Kagaku*, **17**, 24, 52 (1947), *Kagaku-no-Ryoiki* (*Journal of Japanese Chemistry*) **6**, 311 (1952), S. Mizushima, T. Shimanouchi, M. Tsuboi, T. Sugita and E. Kato, *Nature*, **164**, 918 (1949).
6) E. J. Ambrose and A. Elliott, *Proc. Roy. Soc.*, **A 205**, 47 (1951).
7) W. T. Astbury, *Nature*, **164**, 439 (1949).
8) L. Bragg, J. C. Kendrew and M. F. Perutz, *Proc. Roy Soc.*, **A 203**, 321 (1950).
9) L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci.*, **37**, 205, 241 (1951).

$\alpha_{(2)}$, exists in T (Trans) form. Other situations are equal exactly, so $\alpha_{(1)}$ and $\alpha_{(2)}$ can be said to be rotational isomers. If the energy difference between these isomers is small enough, it becomes necessary to consider the equilibrium between them in solution. According to previous experiences, T form is slightly stabler than G form except in special cases. This relation predicts that the equilibrium is shifted towards $\alpha_{(2)}$.

In the case of the polypeptide chain, however, we must take into account not only the above-mentioned interaction between the side chain and the main chain, but also the interaction between the side chain and the C=O group. In $\alpha_{(1)}$, the relative orientation between the side chain and the C=O group comes half way between G and T forms, and in $\alpha_{(2)}$, it exists in C (Cis) form. That is, C=O group takes C form with hydrogen atom on α-carbon atom in the case of $\alpha_{(1)}$, and with side chain (methyl group) in the case of $\alpha_{(2)}$. Consequently, the repulsive energy due to steric hindrance becomes greater in $\alpha_{(2)}$ than in $\alpha_{(1)}$, and $\alpha_{(2)}$ is unstabilized by this amount. Thus this repulsive energy tends to cancel the stabilization energy due to T-type configuration between the side chain and the main chain in $\alpha_{(2)}$. Though one cannot give any conclusion as to whether this cancellation is complete or not, one can expect that the energy difference between $\alpha_{(1)}$ and $\alpha_{(2)}$ is small enough.

In the following calculations, we assume as the idealized limiting case that $\alpha_{(1)}$ and $\alpha_{(2)}$ have the same stability and exist in solution with equal statistical weight. By this assumption, any unknown quantity concerning the equilibrium constant is excluded. Moreover, the main chain of polypeptide comes to have an effective plane of symmetry in the statistical meaning. Consequently, the calculations are exceedingly simplified. Later, some conclusion will be given for a case where the equilibrium is shifted towards $\alpha_{(2)}$ by the analysis of the results of calculation.

Next, we should consider the equilibrium between folded α-type configuration and that of the extended β-type or intermediate ones. In the case of water solution, the stability of the intramolecular hydrogen bond is diminished by some amount, and one can expect and larger contribution of extended or intermediate forms compared with the case in which a non-polar solvent is used. As can be easily seen, the latter ones (β- and intermediate types) have a larger degree of freedom compared with the former one (α-type), and corresponed to the state of larger entropy. Therefore the contribution of latter ones may increase rapidly with the elevation of temperature. Concerning this point, Kauzmann and Eyring suggested that the investigation of the effect of temperature on the molecular rotations of these polypeptidies would be of interest here.

In the actual calculations, the contribution of the latter ones are neglected. So it must be noticed that the calculated results correspond to the experimental results which are observed at sufficientry low temperature. Even if the contribution of latter ones could not be neglected, we might expect that the observed change of molecular rotations in a series of polypeptides would correspond well to the calculated results so long as the equilibrium constant was approximately constant regardless of the length of chains and the molecular rotations of the latter ones were sufficiently small as were inferred by Kauzmann and Eyring.

To simplify the calculations, it is assumed that the interatomic distances in C—C and C—N bonds are all 1.54 Å, and that in C=O and C—H bond is 1.26 Å and 1.1 Å respectively. Further, it is assumed that the valence angles of C and N atoms are tetrahedral angle (109°28′), and C—N bonds (C are not α-carbons) are perpendicular to a dyad axis of symmetry. In the present model, repeat distance is about 5.03 Å. In the actual molecule, true valence angles will make the molecule flatter than the assumed model and the repeat distance slightly larger.

II. Moleculur Rotation

Division of Rotatory Parameter According to the electromagnetic theory, molecular rotation $[M]$ is given as follows,

$$[M]=\frac{288\pi^2N}{\lambda^2}\frac{n^2+2}{3}\beta \qquad (1)$$

where N is Avogadro's number, λ the wavelength of incident polarized light, and n the refractive index of the solution. Taking into account the variation of vector potential over the molecule, Rosenfeld developed a quantum-mechanical theory of dispersion, and gave the following expression for a rotatory parameter β:

$$\beta=\frac{c}{3\pi h}\sum_b{}'\frac{\mathrm{Im}\{(a\mid\vec{\mu}\mid b)\,(b\mid\vec{m}\mid a)\}}{\nu_{ba}{}^2-\nu^2} \qquad (2)$$

Here, $(a\mid\vec{\mu}\mid b)$ and $(b\mid\vec{m}\mid a)$ are the matrix components of electric and magnetic moments connecting state a and b; ν_{ba} the frequency of the transition between state a and b, ν the frequency of the incident plane polarized light, c the velocity of light, and h Planck's constant. Then, the theoretical problem of optical activity is merely to calculate Eq. (2) for the actual molecule. In order to proceed with the calculation, Kirkwood introduced a simplifying assumption, that is, electrons were localized in the respective constituent group not only in normal state but also in excited states. By this assumption, one can break up the total electric and magnetic moments into sums over the groups as follows,

$$\vec{\mu}=\sum_i \vec{\mu_i} \tag{3}$$

$$\vec{m}=\sum_k \frac{e}{2mc}\vec{R}_k\times\vec{P}^{(k)}+\vec{m}_k \tag{4}$$

where $\vec{\mu_i}$ is the electric moment of group i, $\vec{p}^{(k)}$ is the electric momentum of group k, and $\vec{m}_k$ is the magnetic moment referred to the center of gravity of group k situated at a point $\vec{R}_k$ from the center of gravity of the molecule as a whole. Substituting these relations in Eq. (2), Kirkwood obtained the following results.

$$\beta=\beta^{(0)}+\beta^{(1)}+\sum_k \beta^{(k)} \tag{5}$$

$$\beta^{(0)}=-\frac{1}{3h}\sum_{i\neq k}\sum_b \frac{\nu_{ba}}{\nu_{ba}^2-\nu_2} \times Re\{\vec{R}_k[(a\mid\vec{\mu}^{(i)}\mid b)\times b\mid\vec{\mu}^{(k)}\mid a)]\} \tag{6}$$

$$\beta^{(1)}=\frac{e}{3\pi h}\sum_{j\neq k}\sum_b \frac{1}{\nu_{ba}^2-\nu^2}\mathrm{Im}\{(a\mid\vec{\mu}^{(j)}\mid b)(b\mid\vec{m}^{(k)}\mid a)\} \tag{7}$$

$$\beta^{(k)}=\frac{e}{3\pi h}\sum_b \frac{1}{\nu_{ba}^2-\nu^2}\mathrm{Im}\{(a\mid\vec{\mu}^{(k)}\mid b)(b\mid\vec{m}^{(k)}\mid a)\} \tag{8}$$

$\beta^{(0)}$ is the term which was taken up and studied by Kirkwood and he considered that it was originated from the dynamical coupling between two electrons in the different groups i and k.[10,11] Not being studied in detail, the term of $\beta^{(1)}$ has usually been neglected expecting it probably to be small, $\beta^{(k)}$ is the term which was studied by Condon, Eyring, Kauzmann, Walter, et al., and they considered that it resulted from the motion of one electron in a dissymmetrical field of each group.[12,13,14]

As is obvious from the above discussion, we should consider both terms of $\beta^{(0)}$ and $\beta^{(k)}$ at least, in the calculations of molecular rotations. Accordingly, we will calculate both terms of $\beta^{(0)}$ and $\beta^{(k)}$, and give our conclusion considering both contributions from one-electron theory and dynamical coupling theory.

One-Electron Effect

a) Partial Rotation of N→E Type Transition When molecular rotation $[M]$ is observed in the visible region, the contribution from partial rotation becomes greater as the frequency of the absorption band (electronic transition), which is responsible for this partial rotation, exists nearer to the frequency of the observed light. If one remembers the factor $1/(\nu_{ba}^2-\nu^2)$ in equation of dispersion, this situation is evident. Consequently, when the special absorption band exists in the longer wavelength region far apart from the other absorptions in the ultraviolet region, most of the observed molecular rotation is determined by the partial rotation which is associated with this special transition.

In polypeptides, we are now investigating, $N\rightarrow E$ type transition in carbonyl group exists in the longest wavelength region. The calculations of the partial rotation associated with this transition have been carried out by Eyring et al. for 3-methylcyclopentanone[15] and benzoin.[16] Following Mulliken's usage, we define the coordinate of C=O group as follows. That is, we attribute the direction of π-orbitals of C and O to x-axis, that of non-bonding orbital of O to y-axis, and that of C—O bond to z-axis. In the first approximation, Eyring et al. assumed that the electron was localized in the oxygen atom throughout the process of $N\rightarrow E$ type transition, and they attributed it to the transition $2p_y\rightarrow 2p_z$. According to the study by McMurry, however, it should be attributed to the transition $2p_y\rightarrow 2p_x$ in the same approximation.[17] In this transition, the electric moment is zero and the magnetic moment is relatively large. (Even if the contribution of π-orbital of C atom is taken into account, this transition is still impossible). Then in order to obtain the non-zero value of $\beta^{(k)}$, the electric moment must have z-component, that is, one must consider the contribution of $3d_{yz}$-orbital at least in the excited state. This requirement is satisfied by using the following wave function.

$$\begin{aligned}\Psi_a&=\Psi 2p_y\\ \Psi_b&=\Psi_{2px'}+C\ \Psi_{3dyz}\end{aligned} \tag{9}$$

Substituting these wave functions in Eq. (8), one obtains

$$\beta(\mathrm{k})=\frac{e}{3\pi h}\frac{C}{\nu_{ba}^2-\nu^2} \mathrm{Im}\{(\Psi_{2py}\ \mu_z\ \Psi_{3dyz})(\Psi_{2px'}\ m_z\mid\Psi_{2py})\} \tag{10}$$

where, C is given by first-order perturbation theory as follows,

$$\begin{aligned}C&=\frac{(\Psi_{2px'}\mid\sum_i V_i\mid\Psi_{3dyz})}{E_{2px'}-E_{3dyz}}\\ &=\frac{1}{E_{2px'}-E_{3dyz}}\sum_i \gamma_x^{(i)}\gamma_y^{(i)}\gamma_z^{(i)}U_i\end{aligned} \tag{11}$$

$$U_i=\int\Psi_{2p'}V_i\Psi_{3d}(Z^{1,(i)}-3X^{12}{}_{(i)}Z_{(i)}')d\tau$$

Here, V_i is the electrostatic perturbing potential from the atom i except the carbonyl group. (In practice, it is sufficient to consider the contribution from the atoms by which the center or plane of symmetry is lost). In the case of neutral atoms, these potentials originate from the incomplete screening of the nuclear charge on account of the extension of electron clouds, and fall off exponentially with the increase of distances from nuclei. Non-diagonal elements U_i of perturbing potentials

10) J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).
11) W. W. Wood, W. Fickett and J. G. Kirkwood, *J. Chem. Phys.*, **20**, 561 (1952).
12) E. U. Condon, *Rev. Mod. Phys.*, **9**, 432 (1937).
13) E. U. Condon, W. Altar and H. Eying, *J. Chem. Phys.*, **5**, 753 (1937).
14) E. Gorin, J. Walter and H. Eyring, *J. Chem. Phys.*, **6**, 824 (1938).
15) W. J. Kauzmann, J. E. Walter and H. Eyring, *Chem. Rev.*, **26**, 339 (1340).
16) T. C. Kwoh and H. Eyring, *J. Chem. Phys.*, **18**, 1186 (1950).
17) H. L. McMurry, *J. Chem. Phys.*, **9**, 231 (1941).

V_i are correlated to D_i by the following relation

$$D_i = -\frac{32}{5}\frac{a_0}{e^2}U_i$$

where the values of D_i are given by Eyring et al. as the function of distance for the cases of hydrogen and carbon atoms, and we use them in the present calculation. (In Eyring's calculation, U_i is approximated by $\int \Psi_{2p}' V_i \Psi_{2s} Z'^3_{(i)} d\tau$. Quantitatively, this approximation gives probably too great values). The evaluation of two center integral $(\Psi_{2p\sigma}' \mid V_i \mid \Psi_{3dyz})$ requires a rotation of the coordinate system in which the new Z-axis coincides with the line joining the center of the perturbing atom and the carbonyl oxygen atom. The terms γ_x, γ_y and γ_z in Eq. (11) are the direction cosines of the above-mentioned line in the old-coordinate system. The values of γ_x, γ_y and γ_z are calculated using the molecular model which is discribed in Part I.

As the value of electric moment, we assume $(\Psi_{2py} \mid \mu_z \mid \Psi_{3dyz}) = 0.22\ e$. This value is obtained by using the experimental absolute intensity ($f \sim 0.03$) of Rydberg series in ethylene.[18] (For carbonyl group, there is no quantitative data concerning intensity). Strictly speaking, as the value of electric moment one should take the smaller value because the above-mentioned intensity corresponds to the total intensity of a few lines in Rydberg series. For magnetic moment, we assume tentatively

$$(\Psi_{2p\sigma}' \mid m_z \mid \Psi_{2py}) = \frac{eh}{4\pi mci}(\Psi_{2p\sigma}' \mid l \mid \Psi_{2p\pi}) = \frac{eh}{4\pi mci}\,0.91$$

If one considers the contribution of π-orbital of carbon atom in the excited state, this value may probably become smaller. Using these values and $n_D = 1.33$, $\nu = 0.509 \times 10^{15}$ sec^{-1} (D line of Na), one can describe the molecular rotation as follows,

18) J. R. Platt, H. B. Klevens and W. C. Price, *J. Chem. Phys.* **17**, 466 (1949).

$$[M]_D = 45.87 \times 10^3 \frac{0.259}{\nu_{0\sigma}^2 \times 10^{-30} - 0.259}$$

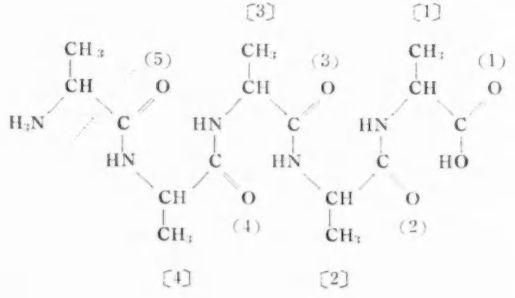
$$E_{2p\sigma}' - E_{3dyz} = \frac{1}{7}\sum \gamma_{x(i)}\,\gamma_{y(i)}\,\gamma_{z(i)}\,D_i \qquad (12)$$

where $\nu_{0\sigma}$ and $E_{2p\sigma}'$ are dependent on the nature of the atom which combines with carbonyl carbon, and use the following values:

	$\nu_{0\sigma} \times 10^{-15}$ sec^{-1}	$E_{2p\sigma}' - E_{3dyz}$ (ev)
H_2N, C=O, C	1.68	−0.58
HO, C=O, C	1.44	−1.58

Of course, these values are not conclusive and may probably become to require some revision in the future study.

b) Method of Calculation We assume the molecular model which is described in Part I. In this model, the rotational isomers $\alpha_{(1)}$ and $\alpha_{(2)}$ have the same stability, and exist in solution with equal statistical weight. Then, the main chain of polypeptide comes to have effective plane of symmetry in the statistical meaning. This situation makes the calculations exceedingly simple, and in order to obtain the partial rotation associated with $N \rightarrow E$ type transition, it is sufficient for it by considering the perturbations from the side chain (methyl group) and the hydrogen atom which is attached to α-carbon together with the side chain. (In the following, we say that the term of methyl group is including this hydrogen atom). As a subsidiary assumption, we consider that end group COOH contributes to the formation of seven-membered ring, and another end group $-CH\langle^{CH_3}_{NH_2}$ exists in the effective plane of symmetry on account of their statistical free rotation around the single bond. Consequently, in case of tetra-alanyl-alanine, the molecular model becomes as follows:



As is shown in the foregoing figure, we number carbonyl oxygens as (1), (2), (3), (4), (5), and methyl groups as [1], [2], [3], [4]. Then, we can divide the perturbations (or partial rotations due to these perturbations) into three classes according to the relative positions between carbonyl oxygen and methyl group, that is:

A: {(1)[1]} {(2)[2]} {(3)[3]} {(4)[4]}
B: {(2)[1]} {(3)[2]} {(4)[3]} {(5)[4]}
C: {(3)[1]} {(4)[2]} {(5)[3]}

As described previously, interaction function D_i decreases very rapidly with the increase of r, and we neglect the interactions at intervals of more than 5 Å (for example {(2)[3]} {(5)[2]} etc.).

In each class of A, B and C, the relative orientations of the carbonyl group and the methyl group are different with the situations whether the configuration of the polypeptide chain exists in $\alpha_{(1)}$ or in $\alpha_{(2)}$. To indicate this distinction, we use the superscripts (1) and (2). In the following, we consider that the above-defined notation, for example $A^{(1)}$, corresponds to the partial rotation due to the perturbation from the methyl group to the carbonyl oxygen which exists in the relative position of class A and configuration of $\alpha_{(1)}$. So long as we assume that $\alpha_{(1)}$ and $\alpha_{(2)}$ are equally probable statistically, the mean value, for example,

$$A=(A^{(1)}+A^{(2)}/2) \qquad (13)$$

corresponds to the observed value. In the present case, the frequency of $N\rightarrow E$ type transition, consequently, the magnitude of the partial rotation depends on the atom, whether it is oxygen or nitrogen, which combines with carbonyl carbon. To distinguish these two cases, we use the subscripts O and N. Then the molecular rotations of a series of polypeptides are given as follows:

$$\left.\begin{array}{ll} \text{alanyl-alanine} & A_O+B_N \\ \text{di-alanyl-alanine} & A_O+A_N+2B_N+C_N \\ \text{tri-alanyl-alanine} & A_O+2A_N+3B_N+2C_N \\ \text{tetra-alanyl-alanine} & A_O+3A_N+4B_N+3C_N \end{array}\right\} \qquad (14)$$

The calculated values of A_O, A_N, B_N and C_N are given in Table II. Perturbations from one

TABLE II

$A_O^{(1)}=110.0°$	$A_O^{(2)}=-22.4°$	$A_O=43.8°$
$A_N^{(1)}=211.5°$	$A_N^{(2)}=-43.1°$	$A_N=84.2°$
$B_N^{(1)}=89.4°$	$B_N^{(2)}=-45.5°$	$B_N=21.9°$
$C_N^{(1)}=44.7°$	$C_N^{(2)}=36.7°$	$C_N=40.7°$

carbon atom and three hydrogen atoms in methyl group and one hydrogen atom attached to α-carbon together with methyl group are all taken into account. Using Eq. (14) and the values in Table II, we can easily calculate the molecular rotations of a series of polypeptides. These calculated values are shown in Table III together with the observed

TABLE III
CALCULATED MOLECULAR ROTATIONS [ ]$_D$, DUE TO ONE-ELECTRON EFFECT

Polypeptides	Calculated values*	Observed values*
alanine	0°	0°
alanyl-alanine	66°	71°
di-alanyl-alanine	213°	192°
tri-alanyl-alanine	359°	360°
tetra-alanyl-alanine	506°	507°

* In the calculated values, $[M]_D$ of alanine is taken as the origin, and corresponding to this situation, observed values are corrected by amount of $[M]_D$ of alanine (13°).

values. As is seen in Table III, the calculated values agree well with experimental ones. It is interesting to see that each of A_O, A_N, B_N and C_N has the same sign, and this is the main reason which enables us to obtain the above-described agreement. If they had different signs, they might cancel with each other in Eq. (14) and give us much smaller molecular rotations.

We must emphasize, however, that the above-obtained quantitative agreement is only an apparent one. Firstly, as is described in the last section, the values of matrix elements U_t, $(\psi_{2py}\ \mu_z\ \psi_{3dyz})$ and $(\psi_{2px}'\ m_z\ \psi_{2py})$ belong to rather upper limits. Secondary, we can expect the appearance of the first member of Rydberg series $(2p\rightarrow 3d)$ in the slightly shorter wavelength region than the position of $N\rightarrow E$ type transition. And the theoretical consideration predicts that the partial rotation associated with this Rydberg transition has the opposite sign to that of $N\rightarrow E$ type transition, and tends to cancel it. Considering this fact, the calculated values become smaller than 20% of the observed values. (Even if we consider the contribution of $4d$, $5d$,.....functions, analogous situations are probable). The above situation is rather inferable, if one remembers that the wavelength of $N\rightarrow E$ type transition is shifted considerably towards the shorter side in case of polypeptide. In the present treatment, the partial rotation associated with the transition of the non-bonding electron in the hydroxyl group or nitrogen atoms in the main chain is not considered. The positions of these transitions, however, exist in the shorter side than that of $N\rightarrow E$ type transition, and the degree of cancellation due to the opposite partial rotation associated with Rydberg series may become greater.

At any rate, it is interesting that the theoretical and observed results agree well at least in qualitative tendency, and the

quantitative comparison will be discussed in the next section considering the dynamical coupling effect.

Dynamical Coupling Effect

a) Theoretical Foamula.—Assuming a dynamical coupling between two electrons in groups i and k (that is, a van der Waals type interaction between groups i and k), Kirkwood applied the first order perturbation calculation to Eq. (6). After some algebraic simplification, he obtained the simple expression for $\beta^{(0)}$ as follows[10,11]*:

$$\beta^{(0)} = \frac{1}{6}\sum_{i>k}^{N} \alpha_i\alpha_k\delta_i\delta_k\, G_{ik}\, \vec{R}_{ik}\, (\vec{b}_i \times \vec{b}_k) \qquad (15)$$

$$G_{ik} = \frac{1}{R_{ik}^3}\left[\vec{b}_i\vec{b}_k - 3\frac{(\vec{b}_i \cdot \vec{R}_{ik})(\vec{b}_k \cdot \vec{R}_{ik})}{R_{ik}^2}\right]$$

$$\vec{R}_{ik} = \vec{R}_k - \vec{R}_i$$

where α_i is the mean polarizability of group i, δ_i is anisotropic factor of α_i and b_i is a unit vector defining the optical axis of symmetry of group i. Application of Eq. (15) is limited in the case that each group has a optical axis of symmetry, and δ_i is defined as follows:

$$\delta_i = (\alpha_{11}^{(i)} - \alpha_{22}^{(i)})/\alpha_i \qquad (16)$$

where $\alpha_{11}^{(i)}$ and $\alpha_{22}^{(i)}$ are the components of α_i parallel and perpendicular to $\vec{t}_i$. The above result obtained by Kirkwood can be considered as a quantum mechanical expression for the classical coupled-oscillator theory of Born and Kuhn. The disappearance of Planck's constant h in Eq. (15) indicates the classical character of it.

b) Method of Calculation.—The molecular model is the same as before. Since the main chain has an effective plane of symmetry in a statistical meaning, the most important factor is the term due to the interaction between methyl group and carbonyl group. In the case of dynamical coupling effect, the transition in methyl group can also contribute to the molecular rotation, and the interaction between the main chain and the side chain should also be taken into account. In the present calculation, however, we neglect this term, and the appropriateness for this omission is discussed in the last part of this paper.

Van der Waals type of interaction decreases more gradually than the interaction which is assumed in one-electron theory, so we take into account the classes of D and E together with those of A, B and C.

D: {(1)〔2〕} {(2)〔3〕} {(3)〔4〕}
E: {(4)〔1〕} {(5)〔2〕}

In the present case, there may be no necessity to distinguish between A_O and A_N, and the molecular rotations of a series of polypeptides are given as follows:

alanyl-alanine	$A+B$
di-alanyl-alanine	$2A+2B+C+D+$
tri-alanyl-alanine	$3A+3B+2C+2D+E$
tetra-alanyl-alanine	$4A+4B+3C+3D+2E$

(17)

In order to calculate the values of A, B, C, D and E using Eq. (15), it is necessary to know the values of $\alpha\delta$ of carbonyl group and methyl group. As the value of $\alpha\delta$, Kirkwood used that of respective group as a whole, however, we used that of respective bond, which was obtained by Wang as a result of careful analysis of Stuart's experimental value.[19] The values obtained by Wang are shown in Table IV (Carbonyl group is assumed to have an optical axis of symmetry in the first approximation). As is seen in Table IV, $\alpha\delta$ of C—H bond is much smaller than those of C—C and C=O bonds, so the contribution of it to molecular rotation can be neglected. Consequently, we consider only interactions between C=O bond and C—C bonds which combine methyl groups with the main chain, and $\vec{R}_{ik}$ is considered as a vector which combines the center of gravity of respective bond. The results of calculation are shown in Table V. It is rather surprising that each of A, B, C, D and E has the same sign in Table V. The calculated molecular rotations obtained by

TABLE IV

LONGITUDINAL AND TRANSVERSAL POLARIZABILITIES OF BONDS (10^{25} cc)

Bonds	α_{11}	α_{22}	$\alpha\delta = \alpha_{11} - \alpha_{22}$
C—H	7.2	6.2	1.0
C—C	18.2	0.2	18.0
C=O	19.4	8.0	11.4

TABLE V

$A^{(1)} = 177.2°$	$A^{(2)} = -0.1°$	$A = 88.6°$
$B^{(1)} = 160.2°$	$B^{(2)} = -0.02°$	$B = 80.1°$
$C^{(1)} = 49.95°$	$C^{(2)} = 4.53°$	$C = 27.2°$
$D^{(1)} = 0.024°$	$D^{(2)} = 30.69°$	$D = 15.4°$
$E^{(1)} = 17.16°$	$E^{(2)} = 17.23°$	$E = 17.2°$

TABLE VI

CALCULATED MOLECULAR ROTATIONS $[M]_D$ DUE TO DYNAMICAL COUPLING EFFECT

Polypeptides	Calculated values	Calculated values × 0.591	Observed values*
alanine	0°	0°	0°
alanyl-alanine	169°	100°	71°
di-alanyl-alanine	380°	225°	192°
tri-alanyl-alanine	609°	360°	360°
tetra-alanyl-alanine	837°	495°	507°

* The observed values are corrected by the same way as in Table III.

* The error of signs in reference (10) is corrected in reference (11).

19) Sheng-Nien Wang, *J. Chem. Phys.*, **7**, 1012 (1939).

using Eq. (17) and the values in Table V are given in Table VI together with the observed values. The calculated values are too great compared with the observed values. But the calculated values multiplied by 0.591 agree relatively well with experimental ones. That is, the calculated results agree with experimental results, at least in qualitative tendency.

c) Discussion on the Results.—In the case of dynamical coupling effect, the calculated values are twice as large as the observed ones. As the main causes of this situation, we can consider the following three factors:

(1) The values of parameters, such as $\alpha\delta$, are too large.

(2) The assumption concerning the equilibrium between two rotational isomers $\alpha_{(1)}$ and $\alpha_{(2)}$ is not appropriate. In the actual calculation, it is assumed that $\alpha_{(1)}$ and $\alpha_{(2)}$ are equally probable, however, one can consider the case in which the equilibrium is shifted towards one side.

(3) In the present calculation, only α-type configurations are taken into account, however, one can also expect the cases in which the contribution of β-and intermediate-type configurations are not negligible.

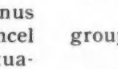
Whether the factor (1) is real or not can be determined by the systematic study for many simple molecules, and at the present stage, it is difficult to give the decided conclusion.

Factor (2) may probably be the most important one, and we will give rather detailed consideration. Observing Tables 2 and 5, one finds that the values of A, B and C are exceedingly larger in $\alpha_{(1)}$ than in $\alpha_{(2)}$. Moreover, the sign of these quantities are definite in $\alpha_{(1)}$, on the contrary, plus and minus signs are mixed in $\alpha_{(2)}$, and tend to cancel each other. The main cause of this situation may probably exist in the fact that the side chains (methyl groups) are approximately perpendicular to the plane of main chain in the case of $\alpha_{(1)}$, on the other hand, side chains are approximately lying in that plane in the case of $\alpha_{(2)}$. If this statement is correct, molecular rotation as a whole considering not only the interaction between side chain and carbonyl group but also all the other interactions in the molecule may be sufficiently smaller in the case of $\alpha_{(2)}$ compared with the case of $\alpha_{(1)}$. Then, the shift of equilibrium towards $\alpha_{(2)}$, makes the molecular rotation of polypeptides smaller. That is, too large theoretical values suggest that the equilibrium is shifted towards $\alpha_{(2)}$ under the observed conditions. This suggestion accords with the expectation from the internal rotation. It is difficult, however, to explain the observed rotations if one neglects the contribution of $\alpha_{(1)}$ completely.

Factor (3) should also be considered, and to know the degree of contribution of this factor, it is very desirable to observe the temperature effect on the molecular rotation.

Factors (2) and (3) diminish not only the molecular rotation due to dynamical coupling effect but also due to one-electron effect as well. Since both effects predict the same sign of molecular rotations (See Table III and VI), that situation has special importance.

Lastly, we consider the degree of approximation of the present calculation. As is described in the last section, only the interaction between the side chain and the carbonyl group is taken into account, and the other interactions, for example, between the main chain and the side chain, are neglected. In order to allow this approximation, it is required that C—C bond and C—N bond (N atom has non-bonding electrons!) have the same $\alpha\delta$. As far as we know, there are no experimental results which are sufficient to verify the above-requirement. We can infer, however, that there will be no large difference between $\alpha\delta$ of C—C and C—N bonds considering the almost equal nature of both bonds. Even if the above-requirement is satisfied, it remains the contribution from the interaction between the side chain and the main chain in the case of di-alanyl-alanine and tetra-alanyl-alanine. We can expect, however, that this contribution may be small if we remember the relatively small values of D and E.

In every case, the influence of the end group  is neglected. Strictly speaking, these omissions may not be reasonable. If we take out the differences of molecular rotations between successive polypeptide molecules, however, the influence due to the end group may mostly be subtracted. We can expect an analogous tendency for the part due to interaction between the side chain and the main chain.

Summary

Molecular rotations of a series of polypeptides of alanine have been calculated theoretically. We have assumed the molecular model which is constructed by seven-membered rings due to intra-molecular hy-

drogen bonds (α-type configuration). Calculations are carried out for the both effects of one-electron theory and dynamical coupling theory, and the results correspond relatively well to experimental results. This fact probably indicates the appropriateness of the assumed model. In order to obtain a decisive conclusion, however, it is necessary to proceed with the calculations for other models and compare the results quantitatively. This is a task for future study.

The author wishes to express his sincere thanks to Prof. S. Akabori for his kind guidance and encouragement throughout this work.

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Equilibrium of the CO_2–NH_3–Urea–H_2O *System under High Temperature and Pressure. V. Liquid-Vapor Equilibrium in the Presence of Excess Ammonia or Carbon Dioxide*[1]

By Schōhachi KAWASUMI

(Received November 4, 1953)

The liquid-vapor equilibrium compositions in a reaction vessel for urea synthesis from ammonia and carbon dioxide under high temperature and pressure have previously been shown in the stoichiometrical loading mole ratio of $2NH_3$ to 1 CO_2[2], assuming that the system consists of three components, ammonia, carbon dioxide and water, in the vapor phase and of four components, urea, water, unconverted ammonia and carbon dioxide in the liquid phase.

For the purpose of presenting similarly the compositions of the liquid and vapor phases of the equilibrium system starting with a mixture of ammonium carbamate and excess amount of ammonia or carbon dioxide in this paper, the yield of urea and the mole ratios of unconverted ammonia to carbon dioxide in the liquid phase of the system have been measured at a temperature of 160°C. The compositions of the system have been calculated from the present and the previous measurements[2] over the range of the loading NH_3-CO_2 mole ratio (L) from 1.6 to 3.0.

On the basis of these results, the effects of the variation of the loading mole ratio on the equilibrium compositions and the equilibrium constant of the following reaction have been discussed.

$$CO_2+2NH_3 \rightleftarrows CO(NH_2)_2+H_2O \quad \cdots\cdots(1)$$

Relation between Loading Composition and Equilibrium Yield of Urea. It is an established fact that the excess ammonia over the stoichiometrical 2 moles per mole of carbon dioxide causes an increase of the equilibrium yield of Urea (Y_e) based on the quantity of the initially charged carbon dioxide. The present measurements of the urea yield under conditions where the excess ammonia or carbon dioxide was added to the carbamate, which was loaded with a constant density of 0.60 g./cc., are plotted against the reciprocal of L in Fig. 1.

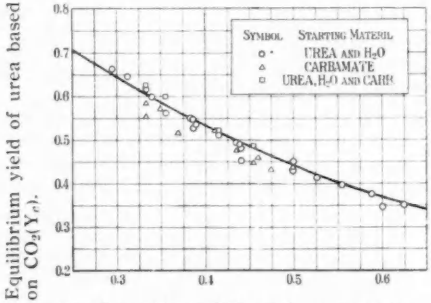

Fig. 1.—Y_e—1/L relation at 160°C. and D=0.60 g./cc.

Y_e was measured by the following procedure: after the system reached the equilibrium, the reaction vessel was rapidly cooled and then the content was withdrawn. Water and unconverted NH_3 and CO_2 were completely evaporated out of the content at 70°C. and the amount of residual urea was weighed.

The urea yield (Y_a) based on the quantity

1) This paper was presented at the annual meeting of the Chemical Society of Japan held at Kyoto University, on April 2, 1953.
2) S. Kawasumi, This Bulletin, **25**, 227 (1952).
3) S. Kawasumi, ibid., **26**, 222 (1953).

of the initially charged ammonia, which is connected with Y_c by the equation: $Y_a \times L = 2Y_c$ is approximately constant independent of L over the range of $L<2$, while it decreases with the rise of L over the range of $L>2$.

It is a remarkable fact that the Y_c-L relation at a constant temperature and loading density depends upon the definition of the loading density. In the present experiments as mentioned above, the starting materials were prepared by loading ammonium carbamate or equi-molar urea and water into the reaction vessel with a constant loading density (D), followed by the addition of the required amount of ammonia or carbon dioxide to it. Consequently, the total loading density (D_t) including excess ammonia or carbon dioxide was varied over the range of L. Regarding the molecular weights of NH_2COONH_4, NH_3 and CO_2 as 78, 17 and 44 respectively, $D_t = \frac{D}{78}(44+17L)$ over the range of $L>2$ and $D_t = \frac{2D}{78L}(44+17L)$ over the range of $L<2$. In this case, therefore, D_t is the minimum at $L=2$ as shown in Fig. 2.

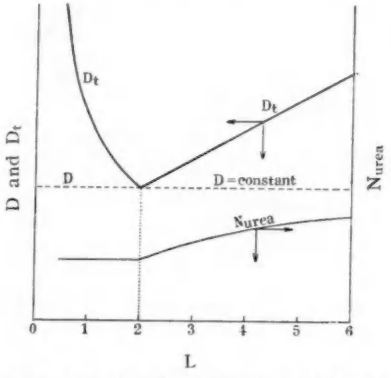

Fig. 2.—D_t—L relation in the experiment performed with a constant D.

If an experiment would be performed with a constant D_t, it would be conjectured that the amount of urea (n_{urea}) in the unit volume of the reaction vessel attains to the maximum at a certain loading mole ratio, since the loading density (D) of the carbamate is the maximum at $L=2$ as shown schematically in Fig. 3. Therefore it may be concluded that the Y_c-L relation at a constant D_t is undoubtedly different from that at a constant D.

The existence of an optimum temperature where Y_c attains to the maximum has already been discussed in the previous paper[2]

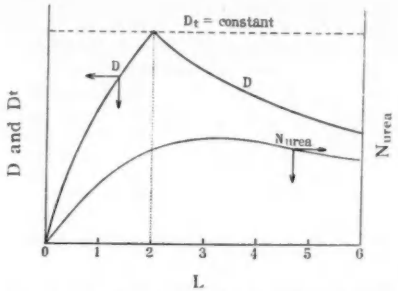

Fig. 3—D—L relation in the experiment performed with a constant D_t.

under conditions where ammonia and carbon dioxide were used in the stoichiometrical proportion to form ammonium carbamate and the loading density of the carbamate was kept constant. Similarly in the presence of excess ammonia or carbon dioxide, the optimum temperature where Y_c attains to the maximum rises with an increase of D at a given L and also it rises with an increase of L at a given D. Such the Y_c-L-T-D relations may be explained to be based upon the same reason as described in the previous paper[2].

Relation between Loading-and Liquid-Composition—Under conditions where carbamate was charged at a loading density of 0.60 g. per cc. of a reaction vessel and then 0.2 to 1.0 moles of excess ammonia or 0.05 to 0.25 moles of carbon dioxide was added to one mole of the carbamate, the mole ratio (R) of unconverted ammonia to carbon dioxide in the liquid phase has been measured at 160°C. by the method reported in the previous paper.[2]

The experimental results are shown in Fig. 4. It has previously been shown that R' increases with L under the same conditions[2]. R also increases with L.

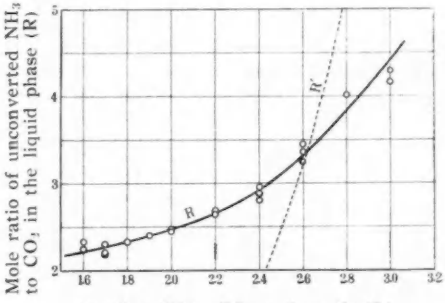

Fig. 4.—R—L relation at 160°C. and D=0.60 g./cc.

Relation between Equilibrium Composition and Loading NH_3-CO_2 Mole Ratio—A method for calculating the equilibrium concentration of each component in the two phases on the same assumption as previously employed[2] is shown in such a case as that where "c" moles of ammonium carbamate (equivalent of "$2c$" moles of ammonia and "c" moles of carbon dioxide) were initially loaded into a reaction vessel of one litre volume and then "m" moles of excess ammonia or carbon dioxide were added to one mole of the carbamate.

Equations giving Concentrations in the Presence of Excess Ammonia—In this case ($L=2+m$), we obtain from the material balance respectively,

$$n_{\text{urea}} + n_{CO_2} + n'_{CO_2} = c \quad \cdots\cdots(2)$$

$$2n_{\text{urea}} + n_{NH_3} + n'_{NH_3} = (2+m)c \quad \cdots\cdots(3)$$

Let us put,

$$n'_{NH_3} / n'_{CO_2} = R' \quad \cdots\cdots(4)$$

$$n'_{H_2O} / n'_{CO_2} = S' \quad \cdots\cdots(5)$$

$$n_{NH_3} / n_{CO_2} = R \quad \cdots\cdots(6)$$

$$n_{\text{urea}} = cY_c \quad \cdots\cdots(7)$$

and

$$n_{H_2O} = n_{\text{urea}} - n'_{H_2O} \quad \cdots\cdots(8)$$

From these seven equations, we can derive the following relations (9) to (14) which allow the equilibrium concentration of each component to be calculated.

$$n'_{CO_2} = \frac{c}{R-R'} \{(1-Y_c)(R-2)-m\} \quad \cdots(9)$$

$$n'_{NH_3} = \frac{cR'}{R-R'} \{(1-Y_c)(R-2)-m\} \quad \cdots(10)$$

$$n'_{H_2O} = \frac{cS'}{R-R'} \{(1-Y_c)(R-2)-m\} \quad \cdots(11)$$

$$n_{CO_2} = \frac{c}{R-R'} \{(1-Y_c)(2-R')-m\} \quad \cdots(12)$$

$$n_{NH_3} = \frac{cR}{R-R'} \{(1-Y_c)(2-R')-m\} \quad \cdots(13)$$

$$n_{\text{urea}} = cY_c \quad \cdots\cdots(7')$$

$$n_{H_2O} = c\left[Y_c - \frac{S'}{R-R'} \{(1-Y_c)(R-2)-m\}\right] \quad (14)$$

Equations giving Concentrations in the Presence of Excess Carbon Dioxide—Similarly in this case ($L=\frac{2}{1+m}$), we obtain the equations (15) and (16) from the material balance.

$$n_{\text{urea}} + n_{CO_2} + n'_{CO_2} = (1+m)c \quad \cdots\cdots(15)$$

$$2n_{\text{urea}} + n_{NH_3} + n'_{NH_3} = 2c \quad \cdots\cdots(16)$$

In the same way from these two equations and the above five equations (4) to (8), we can derive the following relations, for example n'_{CO_2} and n_{CO_2}, which allow the equilibrium concentration to be calculated.

$$n'_{CO_2} = \frac{c}{R-R'} \{(1-Y_c)(R-2)+m \cdots\} \quad (17)$$

$$n_{CO_2} = \frac{c}{R-R'} \{(1-Y_c)(2-R')-mR'\} \quad (18)$$

Equilibrium Compositions in the Presence of Excess Ammonia and Carbon Dioxide—The liquid and vapor phase compositions have been calculated from these equations, as shown in Table 1, under the conditions

TABLE I

LIQUID-VAPOR EQUILIBRIUM COMPOSITIONS IN THE PRESENCE OF EXCESS AMMONIA OR CARBON DIOXIDE AT 160°C. AND D=0.60 g/cc

L	1.60	1.80	2.00	2.20	2.40	2.62	2.80	3.00
c, mole/l.	7.685	7.685	7.685	7.685	7.685	7.685	7.685	7.685
excess CO_2, mole/l.	1.921	0.854	—	—	—	—	—	—
excess NH_3, mole/l.	—	—	—	1.537	3.074	4.764	6.149	7.685
P, atm.	151	114	89	76	66	69.5	75	80
R′	0.156	0.287	0.547	1.035	1.850	3.380	5.350	9.250
S′	0.025	0.040	0.066	0.110	0.175	0.260	0.375	0.575
R	2.220	2.330	2.460	2.618	0.925	3.380	3.827	4.390
Yc	0.355	0.398	0.442	0.483	0.517	0.551	0.577	.0604
Concentration, mole/l. n'_{CO_2}	2.520	1.664	1.031	0.579	0.346	0.203	0.136	0.084
Concentration, mole/l. n'_{NH_3}	0.393	0.478	0.564	0.600	0.639	0.684	0.730	0.781
Concentration, mole/l. n'_{H_2O}	0.063	0.067	0.068	0.064	0.061	0.053	0.051	0.049
Concentration, mole/l. n_{CO_2}	3.676	3.474	3.257	3.395	3.364	3.247	3.114	2.959
Concentration, mole/l. n_{NH_3}	8.150	8.100	8.012	8.887	9.854	10.980	11.915	12.990
Concentration, mole/l. n_{urea}	3.410	3.400	3.397	3.710	3.975	4.235	4.435	4.640
Concentration, mole/l. n_{H_2O}	3.347	3.334	3.329	3.646	3.915	4.182	4.384	4.591
Equilibrium mole fraction N'_{CO_2}	0.874	0.754	0.620	0.466	0.330	0.216	0.149	0.092
Equilibrium mole fraction N'_{NH_3}	0.132	0.216	0.339	0.483	0.612	0.728	0.795	0.855
Equilibrium mole fraction N'_{H_2O}	0.021	0.030	0.041	0.051	0.058	0.056	0.056	0.053
Equilibrium mole fraction N_{CO_2}	0.198	0.190	0.181	0.173	0.160	0.143	0.130	0.118
Equilibrium mole fraction N_{NH_3}	0.439	0.442	0.445	0.453	0.467	0.485	0.500	0.516
Equilibrium mole fraction N_{urea}	0.184	0.186	0.189	0.189	0.188	0.187	0.186	0.184
Equilibrium mole fraction N_{H_2O}	0.180	0.182	0.185	0.185	0.185	0.185	0.184	0.182
$K_{N\text{-}(1)}$	0.870	0.911	0.974	0.985	1.000	1.025	1.049	1.073

where the equilibrium was established at 160°C. starting with a mixture prepared by loading ammonium carbamate of $c=7.685$ moles at a constant density of $D=0.60$ g./cc. into a reaction vessel of one litre volume, followed by adding a required excess amount of ammonia or carbon dioxide to the carbamate. Accordingly the total loading densities including excess ammonia or carbon dioxide are more than 0.60 g./cc. at any loading NH_3-CO_2 mole ratio.

With the increase of the excess amount of carbon dioxide added to the carbamate, the equilibrium pressure[3] rises remarkably and also Y_a is approximately constant independent of the excess amount of carbon dioxide as explained above. According to the calculation, these experimental results are undoubtedly based upon the fact that carbon dioxide is increased not in the liquid, but in the vapor phase. The table also indicates that the larger amounts of the excess ammonia initially added to the carbamate mostly exist in the liquid phase, so that the liquid volume may possibly increase with the excess amount of ammonia.

The liquid-vapor equilibrium diagram of the system at 160°C. is represented as a plot of x_i against L in Fig. 5. As shown in this diagram, x_{urea} and x_{H_2O} attain respectively to the maximum, since an increase of the excess amount of ammonia in the original charge is accompanied by an increase of n_{urea} and n_{H_2O} as well as the total sum of moles (X) of every component in both liquid and vapor phases.

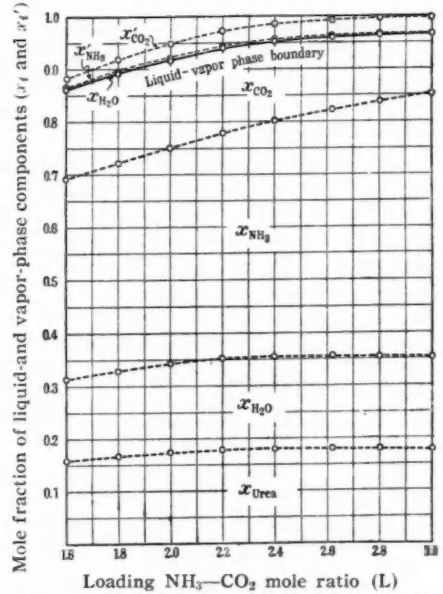

Fig. 5.—Liquid-vapor equilibria diagram of the CO_2—NH_3—Urea—H_2O system in the presence of excess ammonia or carbon dioxide at 160°C.

According to the results of Table 1, the amount of urea is approximately equal to that of water in the liquid phase due to the small amount of water vapor, so that a molecular compound of urea-water, $(NH_2)_2CO \cdot H_2O$, may be assumed for the purpose of expressing the liquid-vapor equilibrium compositions as the three component system, as such an expedient has already been employed by Davis and Black.[4]

In such a manner, a ternary diagram with the apices representing the three constituents—ammonia, carbon dioxide, and water in the vapor or urea-water in the liquid—has also been illustrated from the above data as shown in Fig. 6.

Three pressure-composition diagrams for the systems consisting of every two constituents of them are also given in Fig. 6-A, -B and -C. At a temperature of 160°C. the system exerts the minimum pressures at a point "a" on a vapor curve where the vapor is composed of ammonia and carbon dioxide in the stoichiometrical proportion to form ammonium carbamate, or $R'=2$, and this point is in equilibrium with a point "b" on a liquidus curve which has the composition of $R=2.925$. The coexisting two points of "c" on the vapor curve and "d" on the liquidus curve have the same mole ratio of ammonia to carbon dioxide, that is, $R=R'=3.38$. Usually in the binary system an azeotropic mixture in which the coexisting liquid and vapor have the same composition exerts the maximum or minimum pressure at a constant temperature. It will be seen in this diagram that "a" and "b" are not necessarily consistent with "c" or "d" in the system of three or more components such as the present system.

Further consideration will be given to the point where the coexisting liquid and vapor have the same composition. At this point,

$$R=R' \quad \cdots\cdots(19)$$

By the definitions of L and Y_c and from the material balance,

$$L=\frac{n_{NH_3}+n'_{NH_3}+2n_{urea}}{n_{CO_2}+n'_{CO_2}+n_{urea}} \quad (20)$$

$$Y_c=\frac{n_{urea}}{n_{CO_2}+n'_{CO_2}+n_{urea}} \quad (21)$$

By eliminating all of n_{CO_2}, n'_{CO_2}, n_{NH_3}, n'_{NH_3} and n_{urea} from these five equations of

4) Davis and Black, *Ind. Eng. Chem.*, **23**, 1280 (1931).

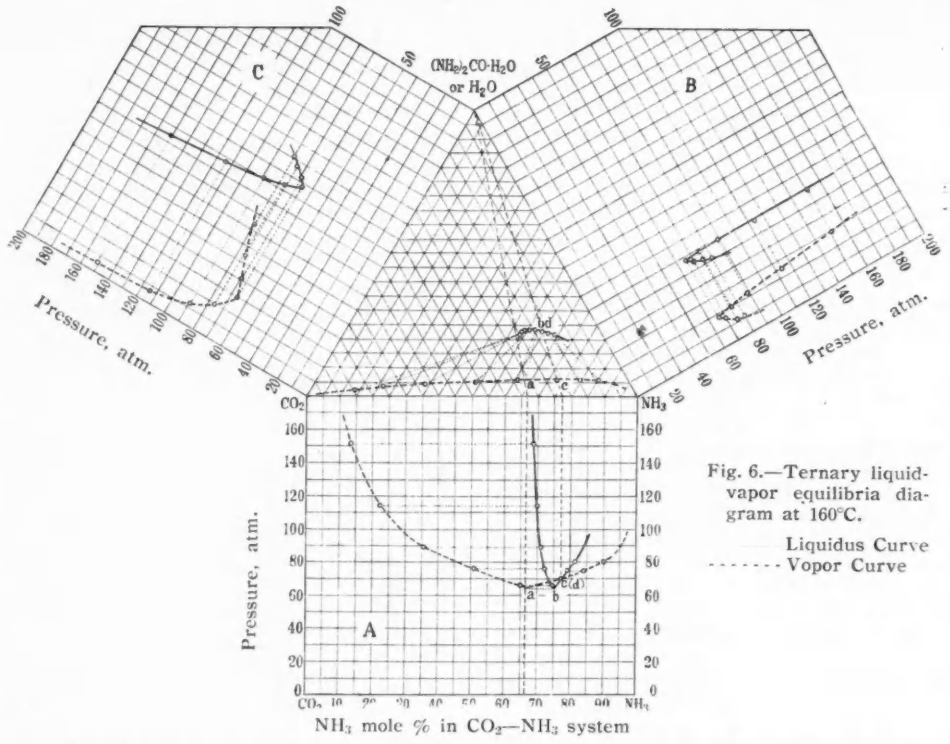

Fig. 6.—Ternary liquid-vapor equilibria diagram at 160°C.

——— Liquidus Curve
- - - - - - - Vapor Curve

(4), (6), (19), (20) and (21), the following relation is derived:

$$L-2=(1-Y_c)(R-2)=(1-Y_c)(R'-2) \quad (22)$$

The present experiments under the condition of $T=160$°C. and $D=0.60$ g./cc. showed the observed values of $R=R'=3.38$ and $Y_c=0.551$ at $L=2.62$ to be in accord with the relation represented by the equation (22).

The equilibrium constants ($K_{N-(1)}$) of the reaction (1) at 160°C. represented by mole fraction (N_i) of each component in the liquid phase ($K_{N-(1)}=\frac{N_{urea}\,N_{H_2O}}{N_{CO_2}\,N_{NH_3}^2}$) are also given in the last line of Table 1. As these values show a tendency to increase gradually with L at a constant temperature, $K_{N-(1)}$ is not an equilibrium constant in the true sense of the term as described in that case in which water has been added to mmonium carbamate.[5] The equilibrium constant should be calculated on the other assumption and explained from the other stand-point in further discussion.

Liquid Volume and Densities—Under the conditions of $T=160$°C. and $D=0.60$ and 0.40 g./cc., the liquid-vapor volume ratios in the presence of the excess amount of ammonia have been measured over the range of $L=2.0\sim3.0$ by a method which was described in the previous paper.[2] The percentage of the liquid volume to the vessel volume increases linearly with L as shown in Fig. 7. The liquid and vapor densities of

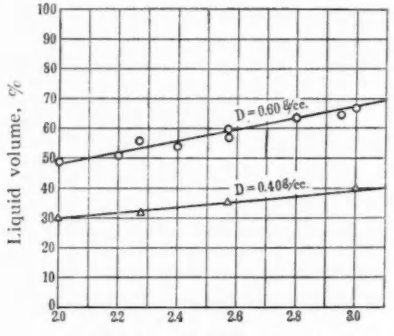

Fig. 7.—Liquid volume in the presence of excess ammonia at 160°C.

the system under high temperature and pressure are given by dividing the weight of each phase obtained from Table 1 by the liquid-and vapor-volume respectively. The

5) S. Kawasumi, This Bulletin, **26**, 218 (1953).

densities (d and d') of the two phases decrease with L, and d′ attains to the minimum at $L=2.7\sim2.8$ as shown in Fig. 8.

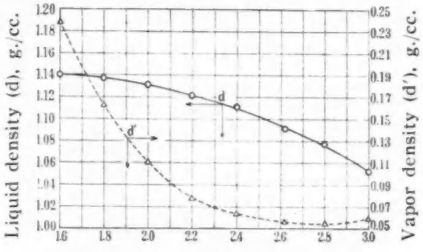

Fig. 8.—Liquid-and vapor-densities over the range of L=1.6~3.0 at T=160°C. and D=0.60 g./cc.

Summary

The liquid-vapor equilibrium compositions of the CO_2-NH_3-Urea-H_2O system starting with a mixture of ammonium carbamate and the excess amount of ammonia or carbon dioxide have been calculated at 160°C. and over the range of the loading NH_3-CO_2 mole ratio of 1.6 to 3.0 from the experimental data presented in the previous paper of this series.

The effects of the loading NH_3-CO_2 mole ratio on the equilibrium compositions have been discussed and the liquid-vapor equilibrium diagram of the system in the presence of excess ammonia or carbon dioxide has been drawn.

Symbols **Nomenclature**

c —Number of moles of ammonium carbamate loaded initially into the reaction vessel, gram mole per litre

d —Density of liquid, gram per cc.

D —Loading density, or ratio of the weights of materials to the total volume of the reaction vessel, gram per cc.

D_t —Total loading density including excess ammonia or carbon dioxide, gram per cc.

$K_{N-(1)}$ —Equilibrium constant of the reaction (1) represented by mole fraction N_i, no units

L —Loading ammonia-carbon dioxide mole ratio, no units

m —Number of moles of excess ammonia or carbon dioxide to one mole of ammonium carbamate, no units

n_i —Number of moles of a component "i" in the liquid phase per litre of the reaction vessel, gram mole per litre

N_i —Mole fraction of a component "i" in the liquid phase, or ratio of gram moles to the total gram moles in the liquid phase, no units

P —Equilibrium pressure, atm.

R —Mole ratio of unconverted ammonia to carbon dioxide in the liquid phase, no units

S —Mole ratio of water to carbon dioxide in the liquid phase, no units

T —Temperature, °C.

X_i —Mole fraction of a component "i" in the liquid phase, or ratio of gram moles to the total gram moles in the two phases, no units

X —Total sum of moles of every component of the two phases, per litre of the reaction vessel, gram mole per litre

Y_c —Equilibrium yield of urea based on carbon dioxide in the original charge, or ratio of gram moles of urea to initial loading moles of carbon dioxide, no units

Y_a —Equilibrium yield of urea based on ammonia in the original charge, no units

Superscript

′(dash) —sign to denote the vapor phase

The writer wishes to express appreciation for the kind guidance and the advice received throughout this work from Dr. Shinjiro Kodama, Prof. of Kyoto University, and for the encouragement received from Dr. Kenji Fujimura, the chief of the Research Department.

Research Department, Niihama Works, Sumitomo Chemical Co., Ltd., Niihama

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On the Relation Between Viscosity and Critical Micelle Concentration of Detergent Solutions. II.[1] *Viscosity and Diffusion Coefficient of Non-ionic Detergent Solutions*[2]

By Haruhiko OKUYAMA and Kiyoshi TYUZYO[3]

(Received November 4, 1953)

Introduction

In the first paper, we proposed the method of determining the first and second critical micelle concentration (CMC) and two kinds of intrinsic viscosity from the analysis of viscosity of various ionic detergent solu-

1) The first paper of this investigation is: N. Sata and K. Tyuzyo, This Bulletin, **26**, 177 (1953).

2) Presented before the sixth Annual Meeting of the Chem. Soc. of Japan in Kyoto, April, 1953.

3) Present Address: Research Laboratory, Dai-Nippon Celluloid Co., Ltd., Sakai City, Osaka, Japan.

tions[1]. In this paper, the results concerning the aqueous solutions of non-ionic detergents are presented. Namely, not only viscosity of the solutions is measured, but also diffusion coefficient is investigated. This latter method is especially effective in determining CMC and molecular weight of micelle in the present case because this method is remarkably simplified on account of negligible action of diffusion potential in the case of non-ionic detergents, in contrast with ionic detergents[4].

Samples and Experimental Methods

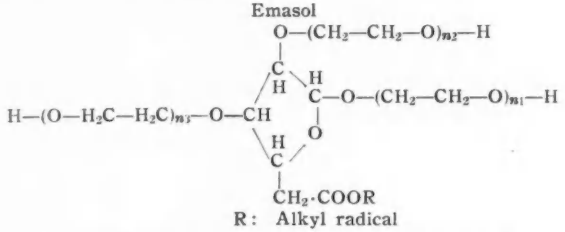
(1) Samples: Several kinds of non-ionic detergents (made by Kao-Soap Co. Ltd.) were used. We used series of polyoxyethylene sorbitan (Emasol) and polyoxyethylene-ether (Emulgen). These have such structures as shown below, and the coexistence of hydrophilic group and hydrophobic group in the same molecule causes the surface activity.

Emulgen

$$R{-}O{-}(CH_2{-}CH_2{-}O)_n{-}H$$

R: Alkyl radical

Emasol



R: Alkyl radical

Pure samples of Kao-Soap Co. Ltd. were used. These contain a small quantity of water, metal salt and polyoxyethylene as the impurities. The content of water is less than 1%[5] and quantity of metal ion is too minute to hinder the measurement of viscosity and diffusion[6]. It was very difficult to remove polyoxyethylene and so we left it as it was.

(2) Viscosity: Viscosity was measured by an ordinary Ostwald's viscometer at 30±0.05°C. The falling time of distilled water was 127.14 sec. Each Sample was measured three times and the averaged value was adopted. But, moreover, at lower concentration (below 1%), measurement was performed with freshly prepared solutions for three times, then the averaged value was obtained.

(3) Partial Specific Volume: Partial specific volume was determined by Ostwald's picnometer of 5 cc content at 30±0.05°C.

(4) Diffusion Coefficient: Diffusion coefficient was measured by the schlieren method using the cylindrical lens as was already shown[7,8]. This time, its accuracy was increased by using the improved diffusion cell. The temperature was 25±0.05°C. The diffusion coefficient at each concentration was obtained by the differential diffusion method[9]. The limiting concentration-gradient of our new cell was about 0.3 weight per cent, but we measured it at the concentration-gradient of 0.5~1% considering the requirement of accuracy.

Results

(1) Partial Specific Volume: The temperature was 30±0.05°C. Partial specific volume of 1% solution is shown in the Table I.

TABLE I. PARTIAL SPECIFIC VOLUME OF 1% SOLUTION OF NON-IONIC DETERGENT AT 30°C

Substance	ρ (g/cc)	V (cc/g)
Emasol 1112	0.9972	0.849
Emasol 1115	0.9964	0.934
Emasol 1120	0.9966	0.916
Emasol 1130	0.9971	0.864
Emasol 3130	0.9978	0.796
Emulgen 120	0.9964	0.934

(2) Viscosity: Fig. 1 shows the relative viscosity-concentration curve of detergents at

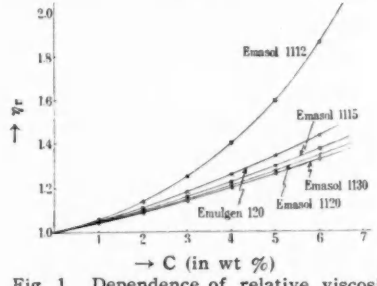


Fig. 1. Dependence of relative viscosity on concentration of several non-ionic detergent solutions.

30±0.05°C. For Emasol 1120, 1130, and Emulgen 120, the η_{sp}/C vs. C-curve becomes minimum at C_1 as is shown in the Fig. 2, but for others, η_{sp}/C simply increases with concentration.

Now, according to the first paper, the second CMC (C_2) and intrinsic viscosity ($[\eta_1]$,

4) G. S. Hartley and C. Robinson, *Proc. Roy. Soc.*, **A134**, 20 (1931).

5) Results of the analysis of water content by Mr. R. Muneyuki of Shionogi-Seiyaku Co., Ltd., were as follows:
Emasol 1112,1.9; Emasol 1115,3.26; Emasol 1120,0.6; Emasol 1130,0.7; Emulgen 120,19.4 mg./g.

6) Results of the measured electric conductivity of several aqueous solutions were as follows:
Emasol 1112, specific electric conductivity of
1% aqueous solution=6×10^{-5}
3% aqueous solution=2×10^{-4}
Emulgen 120, 3% aqueous solution=2×10^{-4}
Potassium chloride 2/10000 N aqueous solution=2×10^{-5}
1/1000 N aqueous solution=1.5×10^{-4}

7) O. Lamm, *Nova Acta Regiae Soc. Scien. Upsal.* IV, **10**, No. 6 (1937); J. S. L. Phillipot, *Nature*, **141** 283 (1938).

8) N. Sata, H. Okuyama, K. Tyuzyo, *Kolloid-Z.* **121**, 46 (1951).

9) L. G. Longsworth, *J. Amer. Chem. Soc.*, **69**, 2510 (1947).

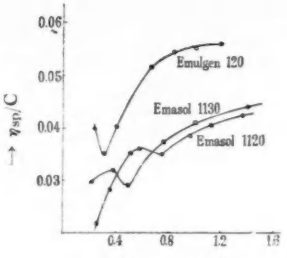


Fig. 2. Relation between η_{sp}/C and C of Emasol 1120, 1130 and Emulgen 120.

$[\eta_2]$ are obtained from the Fig. 3 and the following equation. Namely,

$$\eta_{sp}{}^0/C^0 = \frac{(\eta_r/\eta_{r\,min})-1}{C-C_1} \quad \text{.....(1)}$$

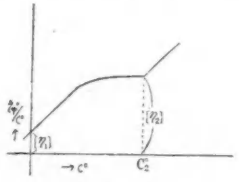


Fig. 3. Determination of C_2, $[\eta_1]$ and $[\eta_2]$.

According to the Equation 1, if we replot the curve for $\eta_{sp}{}^0/C_0$ and C^0, Fig. 3 is obtained. From the curve, the values of C_2 $[\eta_1]$, $[\eta_2]$ are gained.

$$[\eta_1] = \lim_{C^{0}\to 0} (\eta_{sp}{}^0/C^0) \quad \text{.....(2)}$$

$$[\eta_2] = \lim_{C^{0}\to C_2{}^0} (\eta_{sp}{}^0/C^0) \quad \text{.....(3)}$$

$$C_2 = C_1 + C_2{}^0 \quad \text{.....(4)}$$

For the species giving no minimum values of η_{sp}/C, the analogous curve as the Fig. 3 is obtained as is shown in the Fig. 4. Therefore C_2 $[\eta_1]$, $[\eta_2]$ values can easily be gained, although C_2 is not so clear as in the case of ionic detergents except Emasol 1112. But, for the species giving minimum values of η_{sp}/C at C_1, $\eta_{sp}{}^0/C^0$ vs C^0-curve becomes

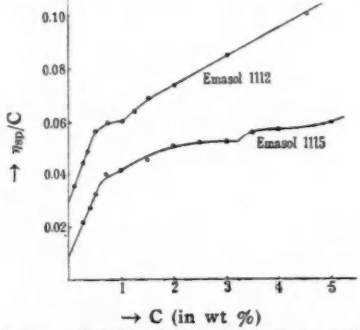


Fig. 4. Relation between η_{sp}/C and C of Emasol 1112 and Emasol 1115.

minimum at $C^0=1$ (Fig. 5). Therefore, $[\eta_1]$, $[\eta_2]$ cannot be obtained. $C^\circ{}_2$ is adopted as the concentration where $\eta_{sp}{}^0/C^0$ vs C^0-curve bends. The above results are summarized in the Tab. II. In the Table, C_1 (Dif.) is the

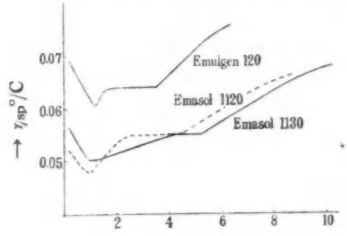


Fig. 5. Relation between $\eta_{sp}{}^\circ/C^\circ$ and C° of Emasol 1120, 1130 and Emulgen 120.

TABLE II CMC OF NON-IONIC DETERGENTS

Substance	C_1 (wt %)	C_2	C_1 (Dif.)
Emasol 1112	0∼0.3	1.10	0∼0.3
Emasol 1115	0∼0.3	3.25	0∼0.5
Emasol 1120	0.75	5.25	0∼2
Emasol 1130	0.50	5.50	0∼1
Emasol 3130	0∼0.3	1.50	.....
Emulgen 120	0.31	3.81	0∼1

concentration where the diffusion coefficient suddenly decreases as will be shown later.

(3) Diffusion Coefficient: In order to examine the accuracy of the diffusion cell, the diffusion of 0.1n potassium chloride solution in water was measured at 20±0.05°C. The diffusion coefficient calculated from the second moment method[10] was 168.4×10^{-7}. This value is satisfactorily consistent with the results of 0. Lamm[11] (166.6×10^{-7}) and Cohen, Bruin[12] (167.5×10^{-7}). As the accuracy of our instrument was found quite satisfactory from the above check-experiment, the following experiments were performed.

As the method of determining the diffusion coefficient from the first and second moment is too laborious and moreover very accurate data are not required in our case, the simplest inflection point method was adopted. Namely,

$$D\mu = \mu^2/2t.G^2 \quad \text{.....(5)}$$

where μ is the x-coordinate at the inflection point of a diffusion curve.

The results are shown in Fig. 6. Diffusion coefficient extrapolated to the zero concentration ($D_0(30)$) at 30°C, calculated from the following equation:

$$D_{T_1}/D_{T_2} = \eta_2 T_1/\eta_1 T_2 \quad \text{.....(6)}$$

where η is the viscosity of solvent, T the absolute temperature provided that the volume of the micelle is independent of temperature, is shown in the Tab. III. It is clear from the Fig. 6 that the diffusion

10) H. Neurath, *Chem. Rev.*, **30**, 357 (1942).
11) O. Lamm, loc. cit.
12) Cohen, Bruin, *Z. Phys. Chem.*, **103**, 347 (1923).

TABLE III DIFFUSION COEFFICIENT EXTRAPOLATED TO THE ZERO CONCENTRATION AT 30°C

Substance	Emasol 1112	Emasol 1115	Emasol 1120	Emasol 1130	Emulgen 120
$D_0(30)\times10^7$	5.92	8.08	8.93	10.1	12.3

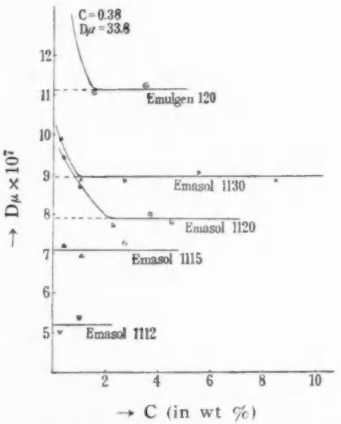


Fig. 6. Dependence of diffusion coefficient on concentration.

coefficient suddenly increases at low concentration for Emasol 1120, 1130, and Emulgen 120, but not for Emasol 1112, and 1115. Moreover, above a certain concentration, the diffusion coefficient is constant and independent of concentration. The concentration region where the diffusion coefficient suddenly increases is shown in the fourth column of Tab. II.

In the next place, the influence of electrolyte on the diffusion was examined. The addition of electrolyte to the aqueous solution of ionic detergent results in the decrease of the diffusion potential and at the same time, in the increase of the micelle size. Therefore, as a result, the diffusion coefficient decreases remarkably. But, for the aqueous solution of non-ionic detergents, the diffusion potential is small and the interaction between electrolyte and hydrophilic group is not so strong as in the case of ionic detergents. Therefore it may be supposed that the influence of electrolyte on diffusion is not so striking as in the case of ionic detergents.

TABLE IV

INFLUENCE OF ELECTROLYTE ON THE DIFFUSION OF NON-IONIC DETERGENT: EMASOL 1112, 1.5 % TO 0.75 %

KCl concentration (mol/l)	$D\mu\times10^7$
0	5.20
0.005	4.90
0.05	4.66
0.2	2.90
0.4	2.75

The used sample was Emasol 1112 and this sample was dissolved in the potassium chloride solutions of various concentrations. Diffusion of 1.5 % to 0.75 % solution was measured at 25°C. Results are shown in the Tab. IV. It is clear from the Table that diffusion coefficients moderately decrease with the increase of electrolyte concentration.

Discussion

(1) It is clear from Tab. II that the concentration at which η_{sp}/C becomes minimum is satisfactorily consistent with the concentration at which the diffusion coefficient suddenly varies. As it is rational that the rapid change of diffusion coefficient is due to the formation of micelle, it can be concluded that the concentration at which η_{sp}/C becomes minimum is CMC even for non-ionic detergents. Therefore the phenomenon that η_{sp}/C becomes minimum at CMC is the common property for both ionic and nonionic detergents and is based not only on the coulombic electrostatic interaction but also on the dipole interaction.

In the next place, it is not clear whether the diffusion coefficient becomes abnormal at C_2 (concentration at which η_{sp}^0/C^0 bends) or not, but at least for Emasol 1130, the diffusion coefficient is constant through C_2 and this fact is different from that of sodium oleate[13].

For Emasol 1112, 1115 (CMC is very low.), η_{sp}^0/C^0 vs C^0-curve is quite analogous to ionic detergents (Fig. 3). On the contrary, Emasol 1120, 1130, and Emulgen 120 show abnormality in the lower concentration region (Fig. 5).

(2) After completion of micelle formation, the diffusion coefficient is constant and independent of concentration. This phenomenon is also found in ionic detergents[14,15]. It is possible that the concentration dependence of diffusion due to the hydrodynamic and thermodynamic interaction[16] compensates with the volume effect. In this case, the diffusion coefficient is apparently

13) H. Okuyama, S. Saito and K. Tyuzyo, Presented before the 4th Annual Meeting of the Chemical Society of Japan in Tokyo, April, 1951. To be published elsewhere.
14) R. J. Vetter, *J. Phys. and Colloid Chem.*, **51**, 262 (1947).
15) O. Lamm, *Kolloid-Z.*, **98**, 45 (1942).
16) S. J. Singer, *J. Chem. Phys.*, **15**, 341 (1947). A. F. Schick and S. J. Singer, *J. Phys. and Colloid Chem.*, **54**, 1028 (1950).

constant in spite of the change of micellar volume. But this possibility is very small and micellar volume may perhaps be constant and independent of concentration.

The region where the diffusion coefficient varies suddenly covers a considerable concentration range. It cannot be concluded from this fact that the process of micelle formation is fairly gradual, because the region where the diffusion coefficient varies suddenly has a moderate width of concentration range even for ionic detergents.

(3) The influence of electrolyte on diffusion is moderately remarkable although not so much as in the case of ionic detergent.[17] The diffusion coefficient of Emasol 1112 in 0.4n potassium chloride solution is one-half what it is in pure water. This means that micellar molecular weight (M_m) becomes about eight times as great. Moreover, Emasol 1112 was dissolved in 1n, 2n, 3n, 4n aqueous solutions of potassium chloride in the concentration of 10 % and was allowed to stand still for 24 hours. Then the 1n solution was almost transparent; the turbidity increased with increasing potassium chloride concentration, the 4n solution being very turbid. This behavior corresponds to the so-called "salting out" phenomenon. From this and above results, it is reasonable to consider that increase of micellar volume and salting out are equivalent phenomena in this case. It seems to be probable that with addition of electrolyte, the hydrophilic property of colloidal particles decreases and they combine with each other, increasing in size and finally they precipitate. We think this concept more probable than that the size is constant and its solubility decreases. It seems to be reasonble to classify "salting out" as follows: (a) Particle size is constant and its solubility decreases. (b) Particle size increases with the increase of electrolyte concentration. This difference may be based upon the degree of separation of hydrophilic and hydrophobic radicals in the same molecule.

(4) Assuming the micelle as a sphere, micellar molecular weight is calculated from the following equation[18]

$$M_m = \frac{(RT)^3}{162\,\pi^2\eta^2 N_A^2 D_0^3 V} \quad \cdots\cdots\cdots(7)$$

where D_0 is the diffusion coefficient extrapolated to the zero concentration, V the specific volume of the micelle which may be substituted by the partial specific volume. Results are shown in Tab. V. These values may have no important meaning, but the values of several ten thousand to hundred thousand suggest that moderately large micelles exist. This corresponds to the fact that CMC is comparatively low and may be based on the weak hydrophilic property of non-ionic detergents compared with ionic detergents.

TABLE V MICELLAR MOLECULAR WEIGHT OF NON-IONIC DETERGENT

Substance	Emasol 1112	Emasol 1115	Emasol 1120	Emasol 1130	Emulgen 120
$D_0 \times 10^7$	5.9	8.1	8.9	10.1	12.3
$M_m \times 10^{-4}$	28.9	10.1	7.8	5.8	3.1

Summary

(1) Partial specific volume, viscosity, diffusion coefficient of the aqueous solutions of non-ionic detergents are measured.

(2) η_{sp}/C becomes minimum at CMC and this concentration is quite consistent with the concentration where diffusion coefficient varies suddenly. It is interesting that this is the common phenomenon for ionic and non-ionic detergents.

(3) The form of η_{sp}^0/C^0 vs C^0-curve is analogous to that described in the first report and the second CMC (C_2) is also obtained but some abnormality occurs in the part of low concentrations for the substance of which CMC is high.

(4) After the completion of micelle formation, diffusion coefficient stays constant and is independent of concentration which suggests that micellar volume is constant and independent of concentration.

(5) The influence of an electrolyte on the diffusion is examined and a new idea of the mechanism of "salting out" is proposed.

(6) Micellar molecular weight is calculated from the diffusion coefficient assuming that a micelle is spherical.

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17) H. Okuyama and K. Tyuzyo, Presented before the Colloid Symposium held by the Chem. Soc. of Japan in Tokyo, November, 1951.

18) H. Neurath, loc. cit.

Sterols and Other Unsaponifiable Substances in the Lipids of Shell Fishes, Crustacea and Echinoderms. XIV. Unsaponifiable Components Other Than Corbisterol in the Lipid of Corbicula

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It was indicated in the 6th report[1] of this series that a di-unsaturated sterol, apparently brassicasterol, and cholesterol in addition to corbisterol occur in the sterols from *Corbicula leana*. The present paper is concerned with a further study on the unsaponifiable components of the lipid of corbicula.

The sterol mixture separated from the unsaponifiable substances of the lipid of corbicula was converted into the steryl acetate mixture, and the latter was subjected to repeated recrystallizations until a small amount of corbisteryl acetate was finally obtained. From the mother liquors of recrystallizations the following three fractions of steryl acetate were recovered: the fraction I of m.p. 125-127°, the fraction II of m.p. 116-119° and the fraction III of m.p. 100-103°.

The fraction I was fractionally crystallized from ethanol, and the least soluble fraction thus obtained was treated with maleic anhydride to remove the component with conjugated double bonds (corbisteryl acetate) completely in form of its maleic anhydride adduct. The portion with no cunjugated double bonds (A) was subjected to a further fractional crystallization by which nine fractions (see Table II) were separated. The lst fraction consisted of an acetate of C_{29} F_2-sterol which was regarded as identical with poriferasteryl acetate. The free sterol from this acetate showed, however, a considerably lower melting point than that reported for poriferasterol. The 8th fraction consisted of an acetate of C_{29} F_1-sterol which was found to be identical with clionasteryl acetate. Hydrogenation of the 2nd fraction gave poriferastanyl (clionastanyl) acetate. These results indicate that the non-conjugated portion (A) consists chiefly of the acetates of poriferasterol and clionasterol. A portion of the fraction I was also fractionated by way of bromide. Debromination of the ether-insoluble bromide yielded a steryl acetate which was recognized as poriferasteryl acetate, but the free sterol obtained by saponification of this acetate had a melting point which was also considerably lower than that reported in the literature. Although it requires a further study on this point, it does not necessarily follow from the lower melting points of the specimens obtained in these experiments that they are inferior in purity.

The fraction II was fractionated by way of bromide, and there was obtained a bromide fraction which, after debromination, gave cholesteryl acetate. The fraction III showed m.p. 111-113° after one recrystallization and was considered to consist substantially of cholesteryl acetate.

Table I records the properties of the sterols obtained in these experiments.

It is seen from the foregoing results that the sterol mixture from corbicula contains poriferasterol, clionasterol and cholesterol in addition to corbisterol, but the presence of the F_2-sterol, apparently brassicasterol, which was found in the sterol mixture from corbicula described in the 6th report, could not be indicated. Hence another steryl acetate fraction obtained from corbicula described in the 8th report[2] was examined. This fraction was recovered from the mother liquors of recrystallizations which were performed to separate corbisteryl acetate from the steryl acetate mixture. It was then treated with maleic anhydride, by which corbisteryl acetate was completely removed in form of its maleic anhydride adduct. The non-conjugated fraction was separated and purified by recrystallization, yielding brassicasteryl acetate of m.p. 150.5-151.5° and $[\alpha]_D^{24} = -63.6°$. Accordingly brassicasterol constitutes a component of the sterol mixture from corbicula described in the 8th report. These facts appear to be ascribed to a very wide variance in the relative contents of poriferasterol, clionasterol and brassicasterol in the sterol mixtures from the different samples of corbicula; the sterol mixture from corbicula described in these experiments contains a relatively large amount of poriferasterol and

1) T. Matsumoto and Y. Toyama, *J. Chem. Soc. Japan*, **65**, 256 (1944).

2) M. Kita and Y. Toyama, *J. Chem. Soc. Japan*, **70**, 451 (1949).

TABLE I

	Free sterol		Acetate		Benzoate	
	m.p., °C	$[\alpha]_D^0$	m.p., °C	$[\alpha]_D$,	m.p., °C	$[\alpha]_D^0$,
C_{29} F_2-sterol from I	141—142	—	147—148	−53.1	140.5—141	−22.2
C_{29} F_2-sterol from I, by way of bromide	145—146	−50.9	146—147	−54.6	—	—
Poriferasterol[2,3]	155—156	−49.7	146.5—147	−53	139.5—140.5	−21.9
C_{29} F_1-sterol from I	137—138	−37.1	135—136	−42.2	133—135	−17.6
C_{29} F_1-sterol from I, by way of bromide	136—137	−38.0	132—133	−40.8	—	—
Clionasterol[2]	137.5—138.5	−37	137	−41.9	134.5—135	−16.8
Stanol from I, obtained by hydrogenation	143—144	+24.5	140.5—141.5	+15.4	—	—
Poriferastanol[2]	143—144	+24.7	140—141	+16.3	—	—
Sterol from II, by way of bromide	145—146	—	114—115	−44.2	—	—
Cholesterol	148	−38	114	−43		

clionasterol but it contains brassicasterol in such a small amount that its presence could not be demonstrated, whereas the sterol mixture from corbicula described in the 8th report, like that described in the 6th report, contains a larger amount of brassicasterol. A similar fact that the content of certain sterol components in the sterol mixture from shell fish of the same species varies widely for each sample of shell fish was noted in a previous study on the sterol components of chiton in which the content of 7-cholestenol differs widely for each sample of chiton.

After separating the sterol mixture from the crude unsaponifiable substances, there was obtained a viscous oily portion (B), which was contaminated with acidic and saponifiable substances. The acetylated product of the portion (B) was subjected to a fractional distillation. There was an indication of decomposition during the distillation, yielding a total distillate in a yield of about 56% of the acetylated product. A higher fraction was saponified, the product was dissolved in acetone, the solution was cooled, and there was obtained a crystalline solid which was found to consist chiefly of a mixture of batyl and chimyl alcohols. Hydrogenation of the remainder of the saponified product gave a small amount of solid material which was considered to contain substantially batyl alcohol. Accordingly the saponified product appears to contain also an unsaturated member (selachyl alcohol) corresponding to batyl alcohol. Lower fractions were saponified, giving a product which contained a methanol-insoluble portion consisting possibly of hydrocarbons. On treating the methanol-insoluble portion with sulfuric acid, the bulk of it was converted into a tarry matter. A closer examination of the components other than alcohols of batyl alcohol series in the viscous oily portion (B) could not be performed in these experiments. However, the unidentified components including hydrocarbons were suspected to contain more or less decomposition products which might have been formed in the course of distillation.

Experimental

Preparation and Fractionation of Steryl Acetate Mixture.—Boiled meat (37.75 kg.) of corbicula was vacuum-dried at 65-75°, giving 11.44 kg. of dried material, from which 1.13 kg. of lipid was extracted with trichloroethylene.* The lipid was saponified with alcoholic potash, the diluted soap solution was treated with ether in the usual way, and there were obtained 188 g. (16.6% of lipid) of unsaponifiable substances. These were crystallized from 3.5 litres of methanol, affording 113 g. of crystalline solid (crude sterol mixture) of m.p. 132-135°. The steryl acetate mixture prepared from crude sterol was subjected to repeated recrystallizations from ethanol by which 0.3 g. of corbisteryl acetate, m.p. 151.5-152°, was separated. Concentration of the mother liquors from the 3rd and subsequent recrystallizations, the 2nd recrystallization and the 1st recrystallization gave the following three fractions of steryl acetate, respectively: (I) 42 g., m.p. 125-127°; (II) 17.2 g., m.p. 116-119°; (III) 7.2 g., m.p. 100-103°.

Fractionation of Fraction I.—A portion (32 g.) of the fraction I was subjected to a further fractional crystallization from ethanol, by which

3) F. R. Valentine, Jr. and W. Bergmann, *J. Org. Chem.*, **6**, 452 (1941).

4) W. Bergmann and R. J. Feeney, *J. Org. Chem.*, **14**, 1078 (1949); W. Bergmann, F. H. McTigue, E.M. Low, W.M. Stockes and R.J. Feeney, ibid., **15**, 96 (1950).

* The fatty acid components of this lipid were studied in a previous paper by the present authors. Cf. Y. Toyama and T. Tanaka, *J. Chem. Soc. Japan*, **74**, 1016 (1953).

the least soluble fraction (5.0 g.) of m.p. 138-139° and k_{28^2}=1.14 was obtained. This was heated with maleic anhydride (1.5 g.) and xylene (30 cc.) for 10 hours at 140° in a sealed tube. After distilling off xylene under vacuum, the reaction product was saponified with alcoholic potash, and the unreacted sterol was extracted from the diluted maleate solution with ether. The ether extract (4.6 g.) showed m.p. 135-136° and k_{28^2}=0. The acetate prepared from the ether extract was fractionally crystallized from ethanol and acetone with the results given in Table II.

TABLE II

Fraction	Yield, g.	m. p., °C
1	0.82	147–148
2	0.27	145.5–146.5
3	0.27	145–146
4	0.27	142–143
5	0.39	142–143
6	0.33	139.5–140.5
7	0.48	138–139
8	0.81	135–136
9	0.84	132–133

The first fraction had $[\alpha]_D^{25}$=−53.1°,* saponification value 124.5 and iodine value by the perbenzoic acid method 110.0 (calculated for $C_{31}H_{50}O_2F_2$: saponification value 123.4, iodine value 111.6). Saponification of this fraction gave a free sterol which had m. p. 141–142° after recrystallization from ethanol. The benzoate prepared form the free sterol had m.p. 137–138° (turbid), 140.5–141° (clear) and $[\alpha]_D^{25}$=−22.2° after recrystallization from ethanol.

The 8th fraction had $[\alpha]_D^{25}$=−42.2°, saponification value 123.9 and iodine value by the perbenzoic acid method 58.2 (calculated for $C_{31}H_{52}O_2F_1$: saponification value 122.8, iodine value 55.6).

The free sterol had m.p. 137–138° and $[\alpha]_D^{20}$=−37.1° after recrystallization from ethanol. The benzoate had m.p. 133–135° and $[\alpha]_D^{20}$=−17.6°. The melted benzoate developed a blue-violet color in the course of its solidification.

The second fraction was hydrogenated at 60–70° in glacial acetic acid using palladium black catalyst. The product, recrystallized from ethanol, was negative for the Liebermann-Burchard reaction, and had m.p. 140.5–141.5° and $[\alpha]_D^{20}$=+15.4°. The free stanol had m. p. 143–144° and $[\alpha]_D^{20}$=+24.5° after recrystallization from ethanol.

Fractionation of Fraction I by Way of Bromide.—A portion (10 g.) of the fraction I was dissolved in 150 cc. of ether and brominated at −10–−15°, and the insoluble bromide (1.26 g.) formed was filtered. This bromide, after being recrystallized from chloroform-methanol, had m. p. 190–191° (with decomposition), $[\alpha]_D^{12}$=−42.8°, and Br-content 42.05% (calculated for $C_{31}H_{50}O_2Br_4$ 41.28%). Debromination of this bromide gave a steryl acetate of m. p. 146–147° and $[\alpha]_D^{20}$=−54.6°.

The free sterol had m. p. 145–146° and $[\alpha]_D^{20}$=−50.9° after recrystallization from ethanol. The ether solution separated from the ether-insoluble bromide was freed from the excess of bromine and concentrated under vacuum, and the product was fractionally precipitated by adding methanol to the ether solution. Debromination of the first precipitate (2.6 g.) and recrystallization of the debrominated product gave a steryl acetate having m. p. 132–133°, $[\alpha]_D^{20}$=−40.8°, saponification value 123.9 and iodine value* 59.6. The free sterol had m. p. 136–137° and $[\alpha]_D^{20}$=−38.0° after recrystallization from ethanol.

Fractionation of Fractions II and III.—A portion (12.5 g) of the fraction II was dissolved in 150 cc. of ether and brominated at about −10°, and the insoluble bromide (0.5 g.) formed was filtered. The filtrate was freed from the excess of bromine and concentrated under vacuum, and the bromide was fractionally precipitated by adding methanol to the ether solution. Six precipitates were separated. Debromination of the second precipitate and recrystallization of the debrominated product gave a steryl acetate of m. p. 114–115°, $[\alpha]_D^{12}$=−44.2°, saponification value 131.1 and iodine value 64.6 (calculated for $C_{29}H_{48}O_2F_1$: saponification value 130.9, iodine value 59.2). The free sterol melted at 145–146°. Hydrogenation of the steryl acetate in glacial acetic acid in the presence of platinum black gave a stanyl acetate which had m. p. 107–108° and $[\alpha]_D^{20}$=+11.9°.

The fraction III showed m. p. 111–113° after one recrystallization from ethanol.

Separation of Brassicasterol from Sterol Mixture Described in the 8th Report.—The sterol mixture from corbicula was converted into the steryl acetate mixture, and the latter was repeatedly recrystallized to separate corbisteryl acetate (see the 8th report). From the mother liquors of recrystallization, a steryl acetate fraction (2.5 g.) of m. p. 142–143.5° and k_{28^2}=2.63 was recovered. In order to remove corbisteryl acetate completely from this fraction, it was treated with maleic anhydride. The yield of the non-conjugated fraction was about 0.6 g. due to a loss during the treatment. Recrystallization of the non-conjugated fraction from ethanol and acetone gave a steryl acetate (0.2 g.) of m. p. 150.5–151.5°, $[\alpha]_D^{24}$=−63.6°, saponification value 127.8 and iodine value 109.7 (calculated for $C_{30}H_{48}O_2F_2$: saponification value 127.3, iodine value 115.2). The free sterol from this acetate had m. p. 143.5–144.5° and $[\alpha]_D^{20}$=−60.1° after recrystallization from ethanol.

* All rotations were taken in chloroform.

* Unless otherwise stated, the iodine values were determined by the pyridine sulfate dibromide method.

Components Other Than Sterols.—The methanol filtrate separated from the crude sterol mixture of the unsaponifiable substances contained a dark greenish-brown viscous liquid having acid value 13.3 and saponification value 41.6. This was acetylated, and a portion (35 g.) of fhe acetylated product was fractionally distilled with the results shown in Table III. There was an indication of decomposition during the distillation, and a conspicuous evolution of gas was noticed.

TABLE III

Fraction	b. p., °C/2 mm Hg	Yield, (g.)	n_D^{20}	Saponification value	Iodine value
1	—145	0.7	1.4695	133.8	54.1
2	145—170	0.9	1.4694	132.9	53.2
3	170—200	2.4	1.4671	138.9	48.2
4	200—210	3.8	1.4657	175.7	44.8
5	210—225	9.2	1.4657	187.8	42.8
6	225—227	1.8	1.4757	182.6	51.5
7	227—230	0.9	1.4827	171.8	61.4
Residue and loss		15.3			

The fractions 4, 5 and 6 were refractionated, and a fraction (3.5 g.) of b. p. 220–225°/2 mm Hg was separated. This fraction was saponified, the product was dissolved in acetone, and the solution was cooled with ice. The crystalline solid separated from the solution had iodine value 3.8, and recrystallization of this solid from acetone yielded a product (0.3 g.) of m. p. 56–57° and acetyl saponification value 266.8 (calculated for $C_{19}H_{40}O_3$: 280.1; calculated for $C_{21}H_{44}O_3$ 261.8). The oily liquid recovered from the acetone filtrate was hydrogenated at 60–70° in ethanol in the presence of Raney nickel. The hydrogenation product, after being recrystallized from 90% ethanol had m. p. 53.5–54.5°, iodine value 3.2 and acetyl saponification value 253.6.

The fractions 2 and 3 were saponified. On treating the product (2.0 g.) with 20 cc. of methanol, there was obtained 0.14 g. of methanol-insoluble portion. This was dissolved in 7 cc. of hexane, and the hexane solution was washed with an equal volume of concentrated sulfuric acid. The sulfuric acid layer became colored dark brown, and there was formed a tarry matter between the hexane and sulfuric acid layers. The hexane solution was washed several times with concentrated sulfuric acid followed by successive washing with a dilute caustic soda solution and water. After distilling off hexane from the hexane solution, there remained a small amount (30 mg.) of pale yellow liquid.

Summary

Among the sterol components of the unsaponifiable substances of corbicula, poriferasterol, clionasterol and cholesterol in addition to corbisterol are present. Among the unsaponifiable components other than sterols, the presence of a mixture of chimyl and batyl alcohols is indicated. The unsaponifiable components appear to contain also unsaturated members (selachyl alcohol series) corresponding to batyl alcohol series. Besides these components, there occur some other liquid components which contain possibly hydrocarbons. But these unidentified components appear to contain more or less the decomposition products which might have been formed during the distillation.

The sterol mixture obtained from corbicula described in the 8th report, like the sterol mixture from corbicula described in the 6th report, has been found to contain brassicasterol as a di-unsaturated sterol.

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Intermolecular Charge—Transfer Spectra. III. Organic Molecular Complexes

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In recent years, quantitative experimental studies have been undertaken concerning the absorption spectra and equilibrium constants of organic molecular complexes. We can expect that the theoretical analysis of these results will provide us with more precise information than before for their electronic structure especially for the mutual interactions between two components. In the first and second sections, we will discuss the magnitude of resonance interaction in relation with the intensities of intermolecular charge-transfer spectra. Next, the contribution of non-resonance interactions, such as van der Waals type interaction and various electrostatic interactions will be considered in comparison with the former.

The Relation between Absorption Intensity and Resonance Interaction

As is easily seen, the straightforward and quantitative calculation of resonance energy between two components is very difficult on account of the extreme complexity of the electronic system and uncertainty of the radial part of the constituent atomic wave function. It is not difficult, however, to obtain a formula which gives us a resonance interaction energy in terms of absorption intensity. Since the latter quantity is known experimentally, this formula enables us to estimate the magnitude of the former.

In the first paper of this series[1] (in the following, we refer to I) we summarized the essence of Mulliken's theory of intermolecular charge-transfer spectra in the approximation which neglects the overlap integral S between two components. In the following ,we use the simplified formulae in I, since the neglect of S does not affect the final results. According to Mulliken's theory[2], the wave function of the ground state of any molecular complex $(A\ B)$ is given by

$$\psi_N = a\,\psi_0 + b\,\psi_1 \tag{1}$$

where ψ_0, ψ_1 is defined respectively

$$\psi_0 = \psi(A\cdot B),\quad \psi_1 = \psi(A^- - B^+)$$

In case of loose complex $(a^2 \gg b^2)$, the energy of the ground state becomes as follows,

$$W_N \simeq W_0 - H_{01}^2/(W_1 - W_0) \tag{2}$$

where $-H_{01}^2/(W_1-W_0)$ corresponds to the resonance interaction energy and W_0 includes the exchange repulsive interaction energy together with various non-resonance interaction energies. (The derivation of Eq. (2) and the definition of W_0, W_1 and H_{01} are given in I. See Eqs. (5), (6), (7), (8) and (9) in I). From I(11), it follows

$$H_{01}^2/(W_1-W_0) \simeq b^2(W_1-W_0)/a^2 \tag{3}$$

Further, from I (12), we obtained the relation

$$\mu_{EN}/e = Q_{EN} \simeq ab(\vec{r}_B - \vec{r}_A)$$

or

$$b^2 \simeq Q_{EN}^2/a^2(\vec{r}_B - \vec{r}_A)^2 \tag{4}$$

Eqs. (3) and (4) provide us with the relation

$$H_{01}^2/(W_1-W_0) \simeq Q_{EN}^2(W_1-W_0)/a^4(\vec{r}_B - \vec{r}_A)^2 \tag{5}$$

On the other hand, oscillator strength f_{EN} of transition $(E \to N)$ is given by

$$f_{EN} = (8\pi^2 mc/3h)\nu Q_{EN}^2$$

From this

$$Q_{EN}^2 = (3h/8\pi^2 mc) f_{EN}/\nu \tag{6}$$

where

$$1/\nu = \lambda = ch/(W_E - W_N)$$

In case of loose complex, we can expect the relation

$$W_E - W_N \simeq W_1 - W_0 \tag{7}$$

The combination of Eqs. (5), (6) and (7) provides us with the formula

$$H_{01}^2/(W_1-W_0) \simeq (3h^2/8\pi^2 m) f_{EN}/a^4(\vec{r}_B - \vec{r}_A)^2 \tag{8}$$

If we assume

$$a \simeq 1$$

Eq. (8) is simplified as follows:

$$H_{01}^2/(W_1-W_0) \simeq (3h^2/8\pi^2 m) f_{EN}/(\vec{r}_B - \vec{r}_A)^2$$
$$= 11.5\times 10^{-16} f_{EN}/(\vec{r}_B - \vec{r}_A)^2 ev \tag{9}$$

In this approximation, the resonance energy is proportional to oscillator strength f_{EN} of intermolecular charge-transfer spectra and does not include the energy quentity explicitly.

In order to obtain the absolute value of the intermolecular charge-transfer interaction energy, we should take into account not only the attractive term of Eq. (8) or (9) but also the exchange repulsive term which is included in W_0 in Eq. (2). As can easily be inferred, the factors which enlarge the attractive term through the increase of H_{01} also produce the increase of exchange repulsive term by almost the same amount. Then we can expect that these two terms which have opposite signs and the nearly equal absolute value will cancel each other almost completly. This situation may probably enable us to explain the relatively large absorption intensity and the relatively small heat of formation of iodine-benzene complex.

1) H. Murakami, This Bulletin, **26**, 441 (1953).
2) R. S. Mulliken, *J. Am. Chem. Soc.*, **74**, 811 (1952).

Analysis of the Experimental Results

The absorption spectra and equilibrium constants between two components have been observed for various types of organic molecular complexes by many workers.[3-7] Their results are shown in Table I together with the representative data on halogen-aromatic complexes.[8-11] They emphasized the proportional relation between equilibrium constant K and the donor character of B-component (or the acceptor character of A-component). Since equilibrium constant K is the measure of stability of complex (strictly speaking in case of the same solvent and equal temperature), the increase of K implies the increase of attractive interaction energy. On the other hand, the increase of donor character of B-component or acceptor character of A-component seems to produce the increase of resonance interaction energy through the decrease of $(W_1 - W_0)$. (See Eq. (2)). Considering these facts, they concluded that the intermolecular charge-transfer interaction was contributing to molecular complex formation as the most essential factor.

If this is the case, Eq. (8) or (9) predicts the increase of f_{EN} with increase of K. As is well known, f_{EN} can be related with molar extinction coefficient by the formula.

$$f_{EN} = (mc^2/N\pi e^2) \cdot 10^3 \int \varepsilon_\nu d\nu / \log_{10} e \simeq 4.32 \times 10^{-9}\ \varepsilon_c \Delta\nu \qquad (10)$$

where $\Delta\nu$ is the half-breadth of absorption curve. Then, one has to expect the increase of ϵ_c with increase of K so long as the magnitude of $\Delta\nu$ is approximately the same for respective class of complexes. According to the data in Table I, the experimental results predict the reverse. That is, the increase of K is accompanied with the decrease of ϵ_c without exception.* In order to avoid this contradiction, one has to postulate that the contribution of intermolecular charge-transfer interaction to the total binding energy is rather smalll compared with that of the other types of

TABLE I

EXPERIMENTAL DATA FOR VARIOUS TYPES OF MOLECULAR COMPLEXES

A-Component	B-Component	Solvent	λ_{max} (Å)	ϵ_c	K	(Temperature)	Reference
s-Trinitrobenzene	Benzene	*n*-Heptane	2800	~4000	4.09	(21.5°)	(5)
	Aniline	Chloroform	~4000	~1550	5.1	(22°)	(4)
Oxalyl Chloride	Benzene	Heptane	2800	3333	0.27		(3)
p-Quinone	Benzene	Chloroform	2800~2900	(2800)[a]	0.42	(25°)	(7)
	Phenol	Cyclohexane	3150	1200	(0.93)[b]	(25°)	(6)
	Hydroquinone	0.05 *M* HCl aq. solution	4400	890	(0.97)[b]	(25°)	„
	Dimethylhydroquinone	Cychohexane	4130	370	0.60	(25°)	„
Maleic anhydride	Benzene	Chloroform	2760	3140	0.68	(25°)	(7)
	Anisol	„	3000	2020	0.84	(„)	„
	Dimethylaniline	„	4140	1590	1.5	(„)	„
Iodine	Benzene	Carbon Tetrachloride	2970	15400	1.72	(22°)	(8)
				14000	1.95	(25°)	(9)
Bromine	Benzene	Carbon Tetrachloride	2920	13400	1.04	(25°)	(10)
Chlorine	Benzene			9090	0.33	(25°)	(11)

a Extrapolated value *b* Interpolated value

3) B. D. Saksena and R. E. Kagarise, *J. Chem. Phys.*, **19**, 994 (1951).
4) J. Landauer and H. McConnell, *J. Am. Chem. Soc.*, **74**, 1221 (1952).
5) D. M. G. Lawrey and H. McConnell, *J. Am. Chem. Soc.*, **74**, 6175 (1952).
6) H. Tsubomura, This Bulletin, **26**, 304 (1953).
7) L. J. Andrews and R. M. Keefer, *J. Am. Chem. Soc.*, **75**, 3776 (1953).
8) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).
9) T. M. Cromwell and R. L. Scott, *J. Am. Chem. Soc.*, **72**, 3825 (1950).
10) R. M. Keefer and L. J. Andrews, *J. Am. Chem. Soc.*, **72**, 4677 (1950).
11) L. J. Andrews and R. M. Keefer, *J. Am. Chem. Soc.*, **73**, 462 (1951).

* In order to calculate the absorption intensity f_{EN} or ϵ_c, one should take into account not only the relative orientation of two components but also the following factors: 1) electron density distribution in the molecular orbitals which are essential for the intermolecular charge-transfer spectra 2) the degree of extension of each atomic orbital (for example, $2p$-function of oxygen atom is much reduced in the degree of extension than that of carbon atom). It is easy to show that these two factors make the value of H_{ca} and consequently that of ϵ_c smaller for the substituted benzene complex than that for benzene complex even if the most compact relative orientation (such as Model (α) or (β)—see later discussion) is assumed.

interactions.

Moreover, the molar extinction coefficients ϵ_c of organic molecular complexes are much smaller than those of halogen-aromatic complexes, that is about 1/10 for many cases. This fact seems to indicate to us the smallness of the intermolecular charge-transfer interaction in organic molecular complexes. Even in case of halogen-aromatic complexes, we postulated in I that the contribution of intermolecular charge-transfer interaction is negligibly small compared with that of van der Waals type interaction. Thus, in case of solution at least, we can conclude that the contribution of resonance type interaction in organic molecular complexes may be very small and the increase of K with increase of donor character of B-component may be due to the contribution of some other sorts of interactions.

In case of crystal, however, we cannot give any conclusion as to whether the contribution of intermolecular charge-transfer interaction is very small or predominant. Contrasting to the isolated 1:1 complex in solution, one molecule comes in contact with several partner molecules in case of crystal. Moreover, as was emphasized by Mulliken, intermolecular charge-transfer force has rather sharp orientational effect.[2] Consequently, there may be a case in which the relative orientation of components is governed by this force in crystal.

Non-Resonance Interactions

a) Dipole-Induced Dipole Interaction. It is evident that the van der Waals type interaction is contribution to the binding energy between two components in any molecular complex. According to our opinion, this interaction is most predominant in general. We must notice, however, that van der Waals type interaction is not characteristic for molecular complex but common for every case.

As one of the characteristic interactions, Briegleb emphasized the importance of dipole-induced dipole interaction for the molecular complexes in which A-component is polynitrobenzene and B-component is polynuclear aromatic hydrocarbon.[12] The relatively large dipole moment of NO_2 group and the large polarizability of polynuclear aromatic hydrocarbon seems to be suitable for this type of interaction. Briegleb calculated the magnitude of this interaction energy for some examples assuming the intermolecular distance as 3 Å. A part of his results is shown in Table II together with the observed heat of formation.

It is interesting to observe that the calculated and observed values of p-dinitrobenzene complex are smaller than any other case for the same B-component. This situation can be understood if we consider the relatively great distance between two NO_2 groups and the cancellation of two dipole fields due to the antiparallel orientation of two groups. Assuming the validity of the relation

$$\frac{W\ (s\text{-trinitrobenzene} \cdot \text{benzene complex})}{W\ (s\text{-trinitrobenzene} \cdot \text{anthracene complex})} \fallingdotseq \frac{W\ (p\text{-dinitrobenzene} \cdot \text{benzene complex})}{W\ (p\text{-dinitrobenzene} \cdot \text{anthracene complex})}$$

the magnitude of dipole-induced dipole interaction energy W for the case of p-dinitrobenzene · benzene complex is estimated as 0.57 Kcal/mol. If we replace p-dinitrobenzene with p-benzoquinone, this value may reduce to about 2/3, that is about 0.3~0.4 Kcal/mol on account of the smaller value of dipole moment of CO group than that of NO_2 group.

On the other hand, the magnitude of van der Waals type interaction between two benzene molecules in the most compact orientation is estimated as about 3 Kcal/mol.[12-15] Then, in case of quinone · benzene or quinone · substituted benzene complex, the magnitude of van der Waals type interac-

TABLE II

CALCULATED DIPOLE-INDUCED DIPOLE INTERACTION ENERGY W AND OBSERVED HEAT OF FORMATION ΔH IN $C_2H_2Cl_4$. (IN KCAL/MOL). BOTH DUE TO BRIEGLEB ET AL.[12]

A-Component	B-Component Anthracene	
	W	ΔH in $C_2H_2Cl_4$
o-Dinitrobenzene	3.0	2.9
m-Dinitrobenzene	1.6	1.5
p-Dinitrobenzene	1.45	1.2
s-Trinitrobenzene	4.6	3.6

W of s-Trinitrobenzene-Benzene Complex is 1.8 kcal/mol

12) G. Briegleb, *Z. physik. Chem.*, **31B**, 58 (1936). G. Briegleb, "Zwischenmolekulare Kräfte und Molekülstruktur," Stuttgart, (1937).

13) J. H. de Boer, *Trans. Faraday Soc.*, **32**, 10 (1936).

14) V. Myers, *Phys. Rev.*, **78**, 348 (1950).

15) H. A. Stuart, "Die Strucktur des Freien Moleküls", Berlin, (1952). (See p. 78)

tion may probably be ten times as great as that of dipole-induced dipole interaction. Consequently, we can expect that the relative orientation of both components in these complexes is determined by van der Waals type interaction almost completely, at least in case of solution. As is well known, two molecules which attract each other by means of van der Waals' forces will always have a tendency to orient themselves into a position where the largest possible number of atoms are in contact with each other. Thus for the case of quinone *p*-disubstituted benzene complex, we can expect the relative orientation as in Model (β). (See Fig. 2.)

b) Electrostatic Interaction In 1949, Akabori and Murakami pointed out the importance of electrostatic interaction between two components which have the complementary charge distribution.[16] For example, *p*-benzoquinone and *p*-distituted benzene (in which substituents $X^{(B)}$ are CH_3, OH, NH_2, etc.) has charge distribution as shown in Fig. 1 (1) and (2) respectively on account of

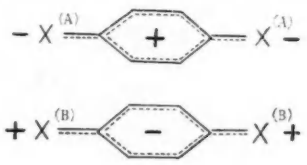

Fig. 1

the resonance effect of π-electron system. If we bring them in the relative position as in Model (β) charges of opposite sign comes nearer than in the case of any other relative orientation. Such a situation as the above suggests the contribution of Coulombic electrostatic interaction together with the van der Waals type interaction. Of course, this type of interaction is characteristic for the molecular complexes and does not exist between the same molecules.

Now we estimate the magnitude of this electrostatic interaction energy by using a point charge model. As simple and practical cases, we consider Model (α) and Model (β) which are shown in Fig. 2. We assume that each substituent $X^{(A)}$ of *A*-component has charge $-q_a$ (in electrostatic unit) and each substituent $X^{(B)}$ of *B*-component has charge q_b (also in electrostatic unit). Further, in order to simplify the calculation, we assume the uniform charge distribution in the benzene ring.

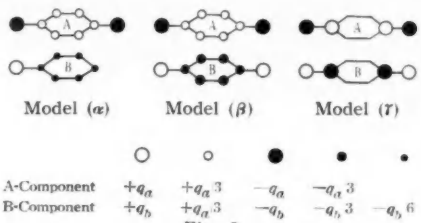

Fig. 2

Then, each carbon atom in the benzene ring of *A*-component has the charge $q_a/3$, and that of *B*-component has the charge $-q_b/6$ in Model (α) and $-q_b/3$ in Model (β). We assume that the intermolecular distance is 3 Å as was taken by Briegleb and the interatomic distances are all 1.39 Å for simplicity. Including all the attractive and repulsive interaction, we obtain the total electrostatic energy $V^{(\alpha)}$ and $V^{(\beta)}$ for Model (α) and (β) respectively as follows:

$$V^{(\alpha)}=V^{(\beta)}/2=-0.100\times10^8 \qquad (11)$$

If we replace q_a and q_b with x_a and x_b which is defined by

$$q_a=\epsilon x_a,\ q_b=\epsilon x_b$$

Eq. (11) becomes as follows:

$$V^{(\alpha)}=V^{(\beta)}/2=-1.45\,x_a x_b \text{ ev}$$
$$=-33.4\,x_a x_b \text{ Kcal/mol} \qquad (11a)$$

As another idealized model, we consider the case in which the charges in benzene ring are concentrated on 1-and 4-carbon atoms with equal proportion (Model (γ)). In this case, the straightforward calculation give the relation

$$V^{(\gamma)}=3V^{(\beta)}/4 \qquad \cdots\cdots(12)$$

Then, we can infer that the actual value is situated between $V^{(\beta)}$ and $V^{(\gamma)}$ in the cases of quinone *p*-disubstituted benzene type complexes.

The remaining task is to evaluate the magnitude of x_a and x_b for the respective molecule. Although the various methods can be adopted for this object, Sklar's method seems most attractive. According to Sklar[17] and Herzfeld[18], x_b of monosubstituted benzne is given by

$$x_b=\sum_j A_j^2 \qquad j=2,3 \qquad (13)$$

$$A_j=\frac{\rho_j-\omega S_j}{\omega-\omega_j} \qquad (14)$$

where

$$\omega=W+(\zeta \mid H_R \mid \zeta) \qquad (15)$$

$$\omega_j=W_j+(\varphi_j \mid H_{X^{(B)}} \mid \varphi_j) \qquad (16)$$

16) S. Akabori and H. Murakami, *Kagaku-no-Ryoiki* (*The Journal of Japanese Chemistry*), **3**, 10 (1949).

17) A. L. Sklar *J. Chem. Phys.* **7**, 984 (1939).

18) K. F. Herzfeld, *Chem. Rev.*, **41**, 233 (1947).

$$\rho_J = \frac{1}{2}\left[S_J(W+W_J)+(\varphi_J \mid \boldsymbol{H}_R+\boldsymbol{H}_{X^{(B)}} \mid \zeta)\right] \quad (17)$$

$$S_J=(\varphi_J \mid \zeta)$$

Here ω_J, ω are the energies of the orbitals in benzene ring R and in substituent $X^{(B)}$ as modified by the coulombic contribution of the other part, that is $X^{(B)}$ and R respectively, S_J the overlap between unoccupied orbital φ_J in R and occupied orbital ζ in $X^{(B)}$, and ρ_J the exchange integral. Numerical results for the cases of $X^{(B)}=OH$ and NH_2 have been carried out by Herzfeld,[18] Nagakura and Baba[19], and $x_b=0.075$ and 0.154 respectively. In case of $X^{(B)}=CH_3$, Ri and Eyring obtained the value $x_b=0.027$ from the rate of nitration of substituted benzene.[20] According to Herzfeld, the effect of more than one substitution ought to be additive as long as the values of A_J are sufficiently small. Then, we use twice these values for disubstituted benzene. (In case of $X^{(B)}=NH_2$, this assumption may be slightly overestimated). The charge distribution of quinone has been calculated by Nagakura and Kuboyama by the molecular orbital method.[21] Their result indicates $x_a=0.503$.

Introducing these values into Eq. (11a), the electrostatic interaction energies for Models (α), (β) and (γ) are easily obtained, and shown in Table III. The increase of interaction energy with increase of donor character of

TABLE III

CALCULATED ELECTROSTATIC INTERACTION ENERGY V IN KCAL/MOL

$X_{(B)}$	Model (α)	Model (β)	Model (γ)
CH_3	0.45	0.9	0.7
OH	1.25	2.5	1.9
NH_2	2.6	5.2	3.9

B-component may enable us to explanin the behavior of equilibrium constant K in Table I. It is evident that these interaction energies are the same order of magnitude as the dipole-induced dipole interaction energies in the case of polynitrobenzene · polynuclear aromatic hydrocarbon complexes.

Appendix

In order to complete the above discussion, we shall consider the correction factors which promote or prevent the electrostatic interaction.

Firstly, we consider the polarization effect as promoting factor. Qualitatively, it is easy to see the increase of charge separation in each component as a result of interaction between complementarily distributed charges. Here, we give a semi-quantitative discussion from the viewpoint of Eqs. (14), (15), (16) and (17). If we express the electrostatic perturbation term from the neighbouring partner molecule by $\boldsymbol{H}'$, ω and ω_J in the perturbed state is given by

$$\omega' = W+(\zeta \mid \boldsymbol{H}_R+\boldsymbol{H}' \mid \zeta) \quad (18)$$

$$\omega_J' = W_J+(\varphi_J \mid \boldsymbol{H}_X+\boldsymbol{H}' \mid \varphi_J) \quad (19)$$

$$\omega'-\omega_J' = (\omega-\omega_J)+\left[(\zeta \mid \boldsymbol{H}' \mid \zeta)-(\varphi_J \mid \boldsymbol{H} \mid \varphi_J)\right] \quad (20)$$

Then, For example, we assume that $\boldsymbol{H}'$ is the electrostatic perturbation from A-component to an electron in B-component. In this case, $(\zeta \mid \boldsymbol{H}' \mid \zeta)$ represents the decrease of effective ionization potential of substituent $X^{(B)}$ and $(\varphi_J \mid \boldsymbol{H}' \mid \varphi_J)$ represents the increase of effective electron affinity of benzene ring. (More strictly speaking, that of orbital φ_J in the ring). Elementary calculation shows that the numerical value of

$$(\zeta \mid \boldsymbol{H}' \mid \zeta)-(\varphi_J \mid \boldsymbol{H}' \mid \varphi_J)$$

is approximated by Eq. (11a) in which $x_b=-1$ and $x_a=0.503$ (the value for quinone). Then, for Model (α), we obtain the relation

$$\omega'-\omega_J' = (\omega-\omega_J)-0.73 \text{ ev}$$

According to Sklar and Herzfeld,

$$\omega-\omega_J = 12.5-7.6 = 4.9 \text{ ev}, \quad (j=2)$$

$$A_J = 0.184$$

for phenol ($X^{(B)}=OH$). By using Eq. (14), these values give us

$$\rho_J-\omega S_J = 0.902 \text{ ev}$$

In the first approximation, we assume that this value is not changed by the electrostatic perturbation. Then, perturbed value of A_2 is given by

$$A_2' = \frac{\rho_J-\omega S_J}{\omega'-\omega_J'} = 0.216$$

The relative change of electron density in the ring is given by

$$(A_2'/A_2)^2 = 1.38$$

We should emphasize that this value is rather the lower limit since the increase of the term $(\rho_J-\omega S_J)$ through the decrease of ω is neglected. It is not unreasonable to expect that the actual value of $(A_2'/A_2)^2$ exceeds 1.5. Analogous effect (though its magnitude may be small) can also be expected for A-camponent. It is evident that these situations contribute to the electrostatic interaction as promoting factor.

Next, we consider the induction effect. For the case of $X^{(B)}=OH$, some workers pointed out that the induction effect in σ-electron system might act as preventing factor.[22,23] This is the case. In OH group, however, the electron affinity of oxygen atom is reduced on account of the relatively large charge transfer from hydrogen into oxygen. Then, one can expect that the amount of σ-electron which is absorbed from neighbouring carbon by oxygen is not so great as is expected

19) S. Nagakura and H. Baba, *J. Chem. Soc. Japan*, **71**, 527 (1950).
20) T. Ri and H. Eyring, *J. Chem. Phys.*, **8**, 433 (1940).
21) S. Nagakura and A. Kuboyama, *J. Chem. Soc. Japan* **74**, 499 (1953).
22) K. Higasi, *Yakugaku Kenkyuu*, **21**, 143 (1949).
23) K. Suzuki and S. Seki, This Bulletin, **26**, 372 (1953).

from the bond moment 0.81 D of C—O bond. As is well known, this bond moment is obtained from the dipole moment of dimethyl-ether and cannot be used as that of C—O bond in C—OH group. In case of $X^{(B)}=NH_2$, electron affinity of the nitrogen atom is much smaller than that of the oxygen atom. Moreover, there are two hydrogen atoms which reduce the effective electron affinity of the nitrogen atom. Accordingly, the induction effect may probably be negligible for C—N bond in $C—NH_2$ group.

The author wishes to express his sincere gratitude to Prof. S. Akabori for his ceaseless interest and encouragement throughout the course of this series.

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On the Diffuse Scattering of X-Rays by Single Crystals of Tetranitromethane[1]

By Tsutomu ODA and Takeo MATSUBARA[2]

(Received January 7, 1954)

Introduction

Oda, Iida, Watanabé and Nitta[3,4,5] reported the crystal structure of cubic tetranitromethane using Laue and oscillation photographic data ($a=7.08$ Å $Z=2$, $T^3_d-I\bar{4}3m$). The observed high symmetry of the crystal, $T^3_d-I\bar{4}3m$, was explained by a statistical arrangement of the atomic groups or molecules within the crystal. In other words, the central carbon atoms of the two molecules in the unit cell lie at the positions 000 and $\frac{1}{2}\frac{1}{2}\frac{1}{2}$, and the eight nitrogen atoms on the four body-diagonals with the C—N bond axes are parallel to each of the other molecules, the orientation of the pairs of oxygen atoms in the nitro groups being, however, in certain disorder around the respective C—N bond axis. The simplest model will be the one in which all the pairs of oxygen atoms rotate freely around the C—N bond axes. A calculation[5]* was carried out based on this model and the result explains well the observed intensities of Bragg reflections and the circular diffuse halo, the maximum occurring at 32° for Cu K_α. In addition to

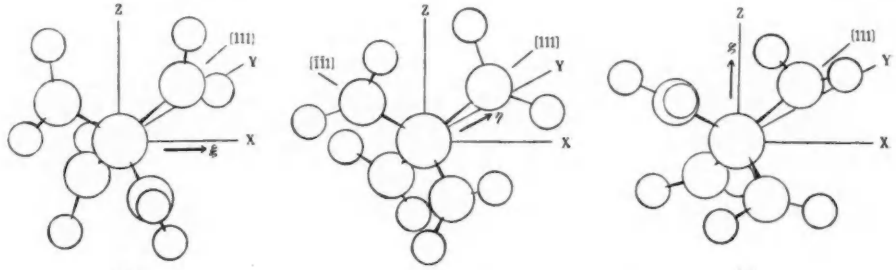


Fig. 1. The three different orientations of molecules in the crystal

1) The main part of this paper was read before the Kansai meeting of the Phyiscal Society of Japan held at Osaka on January 13, 1950.

2) Present address: Department of Physics, Faculty of Science, Hokkaido University, Sapporo.

3) T. Oda, T. Iida and I. Nitta, *J. Chem. Soc. Japan*, **64**, 616 (1943).

4) T. Oda and T. Watanabé, *J. Chem. Soc. Japan*, **65**, 154 (1944). see also; P. H. Lindenmeyer and P. M. Harris, *J. Chem. Phys.*, **21**, 408 (1953).

5) T. Oda and I Nitta, *J. Chem. Soc. Japan*, **65**, 621 (1944).

*) The calculation has given the x-ray diffuse scattering expressed by

$$N(K^\circ-K')=Nf^2_o\{3+\sum_n^{1,2,3,4}2J_0(2c_n)-\sum_n^{1,2,3,4}4J^2_0(c_n)\}, \quad (1)$$

in which N is the number of molecules, f_o the atomic structure factor for oxygen, $J_0(c_n)$ the Bessel function of the zeroth order with the argument c_n, c_n being

$$c_n=4\pi(\rho/\lambda)\sin(\chi/2)\sin(\alpha_n),$$

where χ is the scattering angle, α_n the angle between the reflecting plane and the plane of the two rotating oxygen atoms in the n-th nitro group in the molecule, ρ the radius of the oxygen orbit. It may be added here that this equation is essentially the same as the one (4.237) in Zachariasen's "*Theory of X-Ray Diffraction in Crystals*", 1945 p. 224.

these spot-like diffuse scattering was observed. In this paper we will show a more detailed analysis of the structure taking into consideration the mutual correlation between molecules, which enables us to explain such spot like diffuse scattering.

Theoretical

We shall assume, to simplify matters, a flat-shaped molecule having the symmetry $D_{2d}-\bar{4}2\,m$ (Fig. 1 (a), (b), (c)) which is not very different from the model given by Stosick[6] from his electron diffraction study on gaseous tetranitromethane. We shall take the X-axis in Fig. 1 (a), which is the four-fold alternating axis of the symmetry $D_{2d}-\bar{4}2\,m$, as the molecular axis. It can be shown that by the simultaneous rotation of the four nitro groups, the molecular axis will be brought into Y-(Fig. 1 (b)) or Z-axis (Fig. 1 (c)), the positions of the nitrogen atoms remaining unchanged.

Let us denote by $P_l(x_0x_l)$ the probability that the orientational character of the molecule at the l-th lattice point will be of the kind x_l, when the one at the origin is of the kind x_0. We shall normalize $P_l(x_0x_l)$ as

$$\sum_{x_0}P_{l'}(x_0x_l)=\sum_{x_l}P_l(x_0x_l)=1 \quad .$$

Since, in the present case, x is assumed to possess three possibilities corresponding to molecular orientation in the directions of X, Y and Z axes, we shall designate them further as ξ, η and ζ. As the averaged crystal structure is a body-centered one, there are eight nearest lattice points about the l-th lattice point, which we shall designate as 1, 2, 3,···, μ,···,8. The probability $W(x_1x_2\cdots x_\mu\cdots x_8\,;\,x_l)$ that the l-th is of the kind x_l if the ones surrounding it are of the kinds x_1, x_2,···,x_μ,···,x_8 is now introduced. Then

$$P_l(x_0x_l)=\sum_{x_1}^{\xi,\eta,\zeta}\cdots\sum_{x_8}^{\xi,\eta,\zeta}\prod_{\mu=1}^{8}P_\mu(x_0x_\mu)W(x_1x_2\cdots x_\mu\cdots x_8\,;\,x_l) \qquad \cdots\cdots(2)$$

When correlation is not large, $P_{l'}(x_0x_l)$ will take a finite value, 1/3, for large value of l.

Introducing,

$$P_l(x_0x_l)=(1/3)+R_l(x_0x_l),$$

and using the normalization condition for $W(x_1\cdots x_\mu\cdots x_8\,;\,x_l)$,

$$\sum_{x_1}\cdots\sum_{x_8}\left(\frac{1}{3}\right)^8W(x_1\cdots x_\mu\cdots x_8\,;\,x_l)=\frac{1}{3},$$

it follows that, dropping the suffix zero of x_0,

$$R_l(x\,x')=\sum_{\mu=1}^{8}\sum_{x''}^{\xi,\eta,\zeta}R_\mu(x\,x'')A_\mu(x''\,x')+N_l(x\,x'), \quad \cdots(3)$$

in which

$$A_\mu(x''\,x')=\sum_{x''_1}\cdots\sum_{x''_{\mu-1}}\sum_{x''_{\mu+1}}\cdots\sum_{x''_8}\left(\frac{1}{3}\right)^{8-1}W(x''_1x''_2\cdots x''_{\mu-1}x''\,x''_{\mu+1}\cdots x''_8\,;x'),$$

where $\sum_{x''_1}\cdots\sum_{x''_8}$ means that the summation is to be carried out over three orientations for each of the seven molecules surrounding the l-th lattice point, the μ-th neighbouring molecule being fixed to the orientation x''. In other words, $A_\mu(x''x')$ means the probability that the molecule at the l-th lattice point is of the kind x' when the one at the μ-th neighbouring point is of the kind x''. $N_l(x\,x')$ is the non-linear term of $R_l(x\,x')$.

Expressing the potential energy between two neighbouring molecules by $V_\mu(x''x')$, it is easily shown by symmetry consideration that

$$V(\xi,\xi)=V(\eta,\eta)=V(\zeta,\zeta)$$
$$V(\xi,\eta)=V(\xi,\zeta)=V(\eta,\xi)=V(\eta,\zeta)=V(\zeta,\xi)=V(\zeta,\eta).$$

Since $A_\mu(x''x')$ is expected to be proportional to the Boltzman factor $\exp\{-V_\mu(x''x')/kT\}$, we may assume that

$$A(\xi,\xi)=A(\eta,\eta)=A(\zeta,\zeta)=(1+2\alpha)/3,$$
$$A(\xi,\eta)=A(\xi,\zeta)=A(\eta,\xi)=A(\eta,\zeta)=A(\zeta,\xi)=A(\zeta,\eta)=(1-\alpha)/3,$$

where α is a function depending upon potential and temperature.

Let $\mathbf{b}=(\sigma-\sigma_0)/\lambda$ where λ is the wave length of the x-rays, σ_0 and σ the unit vectors expressing the directions of the incident and scattered rays. The averaged intensity of scattered x-rays will be given by

$$<I(\mathbf{b})>_{\text{Av.}}=\sum_l\sum_{l'}\sum_{x_l}\sum_{x_{l'}}(1/3)P_{l'-l}(x_lx_{l'})f(x_l)f^*(x_{l'})\exp\{2\pi i(\mathbf{b},\mathbf{r}_{ll'})\}, \qquad \cdots\cdots(4)$$

where $f(x_l)$ represents the "molecular" structure factor for the molecule at the l-th lattice point and $\mathbf{r}_l$ its position vector, $\mathbf{r}_{l\,l'}$ being $\mathbf{r}_l-\mathbf{r}_{l'}$. This equation can be put into the following form[7],

$$<I(\mathbf{b})>_{\text{Av.}}=I_1(\mathbf{b})+I_2(\mathbf{b}), \qquad \cdots\cdots(5)$$

in which

$$I_1(\mathbf{b})=\text{Spur}(\boldsymbol{F}\,\boldsymbol{S}_0), \quad I_2(\mathbf{b})=\text{Spur}(\boldsymbol{F}\,\boldsymbol{S}_1), \qquad \cdots\cdots(6)$$

$$\boldsymbol{S}_0=\left(\frac{1}{3}\right)^2\boldsymbol{I}\sum_l\sum_{l'}\exp\{2\pi i(\mathbf{b},\,\mathbf{r}_{ll'})\} \qquad \cdots\cdots(7)$$

$$\boldsymbol{S}_1=\frac{1}{3}N\sum_l\boldsymbol{R}_l\exp\{-2\pi i(\mathbf{b},\,\mathbf{r}_l)\}, \qquad \cdots\cdots(8)$$

$\boldsymbol{I}$ being a matrix whose elements are all one, $\boldsymbol{R}_l$ a matrix whose elements are $R_l(x\,x')$ and N the number of molecules in the crystal. $\boldsymbol{F}$ is a matrix whose elements are products of two "molecular" structure factors for the various orientations. In the present case

$$\boldsymbol{F}=\begin{pmatrix} f_\xi^*f_\xi & f_\xi^*f_\eta & f_\xi^*f_\zeta \\ f_\eta^*f_\xi & f_\eta^*f_\eta & f_\eta^*f_\zeta \\ f_\zeta^*f_\xi & f_\zeta^*f_\eta & f_\zeta^*f_\zeta \end{pmatrix}$$

In (5) I_1 stands for the Laue-Bragg scattering and I_2 for the diffuse scattering. Since $\boldsymbol{R}_l$ tends to zero for large value of l, it is possible[8] to calculate (8) and (6) by an approximate estimation of R_l for small values of l.

From (3) one finds, by introducing matrices $\boldsymbol{A}_\mu$ and $\boldsymbol{N}_l$ whose elements are $A_\mu(x''x')$ and $N_l(x\,x')$ respectively, the following expression,

$$\boldsymbol{R}_l=\sum_{\mu=1}^{8}\boldsymbol{R}_\mu\boldsymbol{A}_\mu+\boldsymbol{N}_l,$$

or, noting that $\boldsymbol{R}_\mu\boldsymbol{I}=0$,

6) A. J. Stosick, *J. Am. Chem. Soc.*, **61**, 1127 (1939).

7) T. Matsubara, *X-Rays*, **6**, 15 (1950).

8) T. Oda, *X-Rays*, **5**, 95 (1949).

$$\boldsymbol{R}_l=\sum_{\mu}\boldsymbol{R}_\mu\boldsymbol{A}'_\mu+\boldsymbol{N}_l, \qquad \cdots\cdots(9)$$

where

$$\boldsymbol{A}'_\mu=\boldsymbol{A}_\mu-\frac{1}{3}\boldsymbol{I}.$$

In the present case, $\boldsymbol{A}'_\mu$ is independent of μ and equal to

$$\boldsymbol{A}'=\frac{\alpha}{3}\begin{pmatrix}2&-1&-1\\-1&2&-1\\-1&-1&2\end{pmatrix}.$$

The summation over l of (9) multiplied by $\exp\{-2\pi i(\mathbf{b},\ \mathbf{r}_l)\}$ gives

$$\boldsymbol{S}_1=\boldsymbol{NM}(\boldsymbol{E}-\boldsymbol{B})^{-1}, \qquad \cdots\cdots(8')$$

where $\boldsymbol{E}$ is the unit matrix, and

$$\boldsymbol{B}=\sum_{\mu=1}^{8}\varepsilon_\mu\boldsymbol{A}'_\mu,\quad \varepsilon_\mu=\exp\{-2\pi i(\mathbf{b},\ \mathbf{r}_\mu)\},$$

$\mathbf{r}_\mu$ being a position vector expressing one of the eight lattice points measured from their central point, and

$$\boldsymbol{M}=\frac{1}{3}\sum_{l}\boldsymbol{N}_l\exp\{-2\pi i(\mathbf{b},\ \mathbf{r}_l)\}. \qquad \cdots\cdots(10)$$

In this case $\boldsymbol{B}$ is given by

$$\boldsymbol{B}=8(\cos\pi H\cos\pi K\cos\pi L)\boldsymbol{A}',$$

where $H=(\mathbf{b},\ \mathbf{a}_1)$, $K=(\mathbf{b},\ \mathbf{a}_2)$, $L=(\mathbf{b},\ \mathbf{a}_3)$ corresponds to a point in the reciprocal space, $\mathbf{a}_1$, $\mathbf{a}_2$, $\mathbf{a}_3$ being the cubic vector set.

By transforming $\boldsymbol{B}$ into the diagonal matrix, $I_2(\mathbf{b})$ is expressed as follows:

$$I_2(\mathbf{b})=N\sum_{i=1}^{3}\frac{m_{ii}}{1-\lambda_i}$$

where $\lambda_1=0$, $\lambda_2=\lambda_3=8\alpha\cos\pi H\cos\pi K\cos\pi L$, and m_{ii} corresponds to diagonal elements of the matrix $\boldsymbol{UFMU}^{-1}$ or $\boldsymbol{UFU}^{-1}\ \boldsymbol{UMU}^{-1}$, $\boldsymbol{U}$ being unitary and

$$U=\begin{pmatrix}1/\sqrt{3}&1/\sqrt{3}&1/\sqrt{3}\\1/\sqrt{2}&0&-1/\sqrt{2}\\1/\sqrt{6}&-2/\sqrt{6}&1/\sqrt{6}\end{pmatrix}$$

which has been used in the diagonalization of $\boldsymbol{B}$. $\boldsymbol{UFU}^{-1}$ is calculated as

$$\begin{pmatrix}(1/3)\ |f_\xi+f_\eta+f_\zeta|^2 & (1/\sqrt{6})\ (f_\xi^*+f_\eta^*+f_\zeta^*)(f_\xi-f_\zeta) & (1/\sqrt{23})\ (f_\xi^*+f_\eta^*+f_\zeta^*)(f_\xi+f_\zeta-2f_\eta)\\ (1/\sqrt{6})\ (f_\xi^*-f_\zeta^*)(f_\xi+f_\eta+f_\zeta) & (1/2)\ |f_\xi-f_\zeta|^2 & (1/2\sqrt{3})\ (f_\xi^*-f_\zeta^*)(f_\xi+f_\zeta-2f_\eta)\\ (1/3\sqrt{2})\ (f_\xi^*+f_\zeta^*-2f_\eta^*)(f_\xi+f_\eta+f_\zeta) & (1/2\sqrt{3})\ (f_\xi^*+f_\zeta^*-2f_\eta^*)(f_\xi-f_\zeta) & (1/6)\ |f_\xi+f_\zeta-2f_\eta|^2\end{pmatrix}$$

If we neglect all higher terms except the one for $l=0$ in (10), $\boldsymbol{M}$ and $\boldsymbol{UMU}^{-1}$ are given as follows:

$$\boldsymbol{M}=(1/3)\boldsymbol{N}_0 \text{ and } \boldsymbol{N}_0=\boldsymbol{R}_0-\sum_{\mu=1}^{8}\boldsymbol{R}_\mu\boldsymbol{A}'.$$

$\boldsymbol{R}_0$ can be written as

$$\boldsymbol{R}_0=\boldsymbol{E}-(1/3)\boldsymbol{I}=\frac{1}{3}\begin{pmatrix}2&-1&-1\\-1&2&-1\\-1&-1&2\end{pmatrix}.$$

Hence, if we take $\boldsymbol{R}_\mu=\boldsymbol{R}_0\boldsymbol{A}'$, $\boldsymbol{UMU}^{-1}$ becomes

$$\boldsymbol{UMU}^{-1}=\begin{pmatrix}0&0&0\\0&m'_{22}&0\\0&0&m'_{33}\end{pmatrix},$$

where $m'_{22}=m'_{33}=(1-8\alpha^2)/3$. From these calculations it follows that

$$I_2(\mathbf{b})=N\left(\frac{1}{3}\right)^2(1-8\alpha^2)\frac{|f_\xi-f_\eta|^2+|f_\xi-f_\zeta|^2+|f_\eta-f_\zeta|^2}{1-8\alpha\cos\pi H\cos\pi K\cos\pi L}, \qquad \cdots\cdots(11)$$

where

$$f_\xi-f_\eta=f_0\{(C_\xi-C_\eta)+i(S_\xi-S_\eta)\},$$
$$f_\xi-f_\zeta=f_0\{(C_\xi-C_\zeta)+i(S_\xi-S_\zeta)\},$$
$$f_\eta-f_\zeta=f_0\{(C_\eta-C_\zeta)+i(S_\eta-S_\zeta)\},$$

and

$$C_\zeta=4\cos2\pi Lz(\cos2\pi Hx\cos2\pi Ky+\cos2\pi Kx\cos2\pi Hy)$$
$$S_\zeta=-4\sin2\pi Lz(\sin2\pi Hx\sin2\pi Ky+\sin2\pi Kx\sin2\pi Hy),$$

f_0 being the atomic structure factor for oxygen atoms and x, y, z its parameters.

Discussion of the Results

When $\alpha=0$, $I_2(\mathbf{b})$ becomes

$$N(1/3)^2\{|f_\xi-f_\eta|^2+|f_\xi-f_\zeta|^2+|f_\eta-f_\zeta|^2\}, \qquad \cdots(12)$$

or

$$N\{(1/3)(|f_\xi|^2+|f_\eta|^2+|f_\zeta|^2)-|(f_\xi+f_\eta+f_\zeta)/3|^2\}.$$

This last equation is equivalent to

$$N\{\langle|f(\boldsymbol{x})|^2\rangle_{\text{Av.}}-|\langle f(\boldsymbol{x})\rangle_{\text{Av.}}|^2\},$$

which was derived in the previous paper[5] dealing with free axial rotation of nitro-groups. These equations account well for the observed halo-type diffuse scattering.

Now we will discuss the case where $\alpha\neq0$. Since there is no long-range order with respect to molecular orientation, the correlation between neighbouring molecules would be small, in other words, the value of α will not be large. So we may assume $1>8\alpha$. The sign of α depends upon the interaction energy $V(xx')$ between two neighbouring molecules. For example if $V(\xi,\ \xi)>V(\xi,\ \eta)$, then $\alpha<0$, and on the other hand if $V(\xi,\ \xi)<(\xi,\ \eta)$, then $\alpha>0$.

Since the function

$$|f_\xi-f_\eta|^2+|f_\xi-f_\zeta|^2+|f_\eta-f_\zeta|^2 \qquad \cdots\cdots(12')$$

changes slowly with $\mathbf{b}$, one finds that the sharp maxima of I_2 $(\mathbf{b})$ occur in the directions for which $1-8\alpha\cos\pi H\cos\pi K\cos\pi L$ possesses minimum value. When $\alpha<0$, such

directions are given by H, K, L with integers and $H+K+L=2n+1$. When H, K, L are integers, we have $H=h$, $K=k$ and $L=l$. However diffuse spots associated with {110}, and {112} (Table I) appeared. Hence, the assumption that α is negative leads to disagreement with the observation, provided that the observed diffuse spots are to be explained only by the correlation of the orientations of molecules, disregarding the influence of thermal motions. On the other hand if we take $\alpha>0$, then the maxima of I_2 (**b**) occur for the directions **b**(corresponding to $h+k+l=2n$), in agreement with the observation.

TABLE I

OBSERVED INTENSITIES OF MAXIMA OF THE DIFFUSE SPOTS WITH THE DIRECTIONS $H=h$, $K=k$ AND $L=l$ AND THEIR CALCULATED VALUES

HKL	Obs.	Calc*
110	st.	29.6
200	w.	0.9
112	st.	19.2
220	w.	12.0

* In the calculation the polarization and the usual temperature fators have been also taken into account, B in $\exp\{-B\sin^2(\chi/2)\}$ being given as 3.

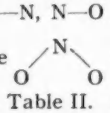
We may assume that the potantial energies $V(x\,x')$ depend primarily upon the interaction between oxygen atoms of neighbouring molecules. The calculation of the distances between these oxygen atoms was carried out, taking the bond distances of C—N, N—O as 1.47, 1.21 Å and the bond angle  as 127°. The results are shown in Table II. In this case, we could expect that the potential energies for the two kinds of mutual orientations would not differ very much, so that $|\alpha|$ would be of a small value. It is, however, difficult to decide conclusively from such a qualitative consideration which one of these two is more stable and has a higher probability.

TABLE II

CALCULATED VALUES OF THE INTERMOLECULAR ATOMIC DISTANCES BETWEEN OXYGEN ATOMS

I	II
2.73 Å	2.92 Å
2.73	3.12
3.69	3.44
3.69	3.67
3.96	3.69
3.96	3.96

I stands for the parallel orientation ξ, ξ and II for the perpendicular ξ, η.

In the theory stated above, it is seen that when $\alpha>0$, the maxima depend upon the values of (12′). In Table I are tabulated the calculated values of this equation, with the parameter values $x=0.28$, $y=0.11$, $z=0.16$ which are close to those $x=0.2675$, $y=0.054$, $z=0.1607$ derived from the bond-lengths and bond-angles as mentioned above. As is seen the calculation explained qualitatively the observed results. Further approximation will be attained by examination of the more appropriate model[4,5] of the molecule having the symmetry S_4—$\bar{4}$ and by taking into consideration of the lattice vibrations[9].

In conclusion the authors wish to express their sincere thanks to Professor I. Nitta, Professor T. Watanabé and Professor T. Nagamiya, Faculty of Science of Osaka University, for their available discussion and kind encouragement throughout this research.

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9) H. Faxén, *Z. Physik*, **17**, 266 (1923). I. Waller, *Z. Physik*, **17**, 398 (1923). W. H. Zachariasen, *Phys. Rev.* **57**, 597 (1940); **59**, 860 (1941). G. H. Beglie and C. M. Born, *Proc. Roy. Soc.* A **188**, 179, 189 (1947).

Perjodat-Oxydation von Ovalbumin

Von Kazuyuki MAEKAWA und Masahisa KUSHIBE

(Eingegangen Am 7, Januar, 1954)

Früher wurden häufig die Oxydationsagenzien wie Permanganate, Wasserstoffsuperoxyd, Chromsäure angewandt, um die Strukture der Eiweissstoffe mit Hilfe von Oxydation zu erklären. Bei diesen Versuchen sind sie jedoch so kräftig, dass verschiedene Stelle an dem Molekül angegriffen und damit die Reaktion kompliziert wird. Ausser bei den verhältnismässig einfachen Eiweissstoffen brachten die solchen Versuche kein ergiebiges Ergebnis[1,2]. Hinsichtlich der Beziehungen zwischen der Strukture der Eiweissstoffe und ihrer physiologischen Wirkung brachte die direkte Oxydation von Eiweissstoffen fast nichts zu Tage.

Dagegen wurden neulich einige Versuche veröffentlicht, native Eiweissstoffe unter verhältnismässig milden Bedingungen zu oxydieren. Goevel et al. fanden z.B., dass Virus, *„Western equine encephalomylitis"*, infolge der Perjodat-Oxydation seine Pathogenität verliert, dabei aber seine antigenische Spezifität zurückbleibt[3]. Ferner folgerten Goebel und Perlmann[4] aus den Veränderungen an Absorptionsspektrum und elektrophoretischer Mobilität, dass sich einige Amino-Säuren zerstören liessen, indem sie mittels $LiIO_4$ Rinde Serumalbumin oxydierten.

Desnuelle et al. berichteten[5], dass Ovalbumin durch Perjodat allmählich niedergeschlagen wurde, und dass sämtlich Cystein, Cystin, ein Teil von Tyrosin und ein Drittel von Tryptophan in dem koagulierten Ovalbumin zersetzt wurden. Vor kurzer Zeit behandelten Jansen et al.[6] mit Perjodat α-Chymotrypsin und gewannen Oxy.-α-Chymotrypsin, welche opt. *p*H mehr oder weniger verschob.

Betreffs Chemismus von Eiweiss-Oxydation befindet sich aber die eingehende Forschung noch nicht.

Schon berichtete einer von uns gemeinsam mit M. Hamada[7], dass verschiedenartige Eiweissstoffe gewissermassen das Oxydant verbrauchten und dabei gewisses Ammoniak und Aldehyd entstanden. Jedoch muss noch erklärt werden, dass dieser scheinbare Perjodat-Verbrauch von Protein verrichtet wurde, um welche Stelle von dem Proteinmoleküle wirklich zu oxydieren, und was Protein folgendessen für eine Veränderung physikalisch erlitt, und auch welche Aminosäuren welcherweise sich zersetzten. Um weiteres darüber zu erklären und zur Kenntnisse der physiologischen Wirkung und Strukture zu kommen, nahmen die Verfasser die folgenden Untersuchungen vor.

Beschreibung der Versuche

I. Direkte Oxydation von kristallizierten Ovalbumin durch Perjodat. Ovalbumin, welches nach gewöhnlichen Verfahren dargestellt, mehrfach umkristallisiert und dialyziert wurde, wurde in Michaelis'schen Phosphat-Puffer von *p*H 7.7 aufgelöst, um 5～20 mg/ccm von Eiweisslösung darzustellen. $NaIO_4$ wurde aus Wasser umkristallisiert und in demselben Puffer von *p*H 7.0, als 0.1～0.01 N Lösung gelöst. Unter Benutzung von beiden Lösungen wurden die Bestimmung von Oxydant-Verbrauch und die Darstellung von oxydierten Proben folgendermassen jede für sich verrichtet.

(A). 10 ccm dieser Ovalbuminlösung wurde in 50 ccm Glasstöpselflaschen pipettiert, hierzu wurde 20 ccm 0.02 N Natriummeta-Perjodat Lösung zugesetzt, diese wurde bei Zimmertemperatur unter gelegentlicher Pipettierung gelassen. 1 ccm von der ausgezogenen Lösung wurde mit 10 ccm gesättigter $NaHCO_3$-Lösung und 1 ccm 10%-iger KJ Lösung versetzt, danach mit 1 ccm 0.5% Stärkelösung, zuletzt mit Wasser bis zu 50 ccm

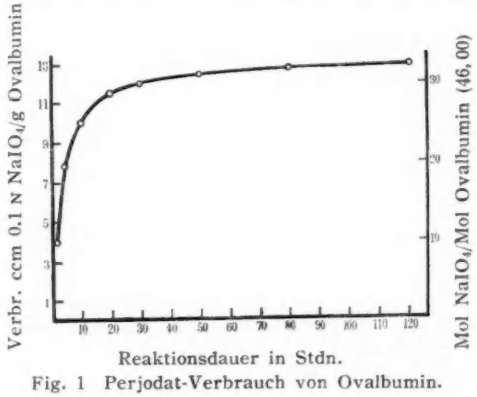

Fig. 1 Perjodat-Verbrauch von Ovalbumin.

1) S. Goldschmidt, und K. Straus, *Ann.*, **480**, 263 (1930).
2) R. M. Herriott, "Adv. in Protein Chem.", **3**, 175 (1947).
3) W. F. Goebel, P. K. Olitsky und A. C. Saenz, *J. Exp. Med.*, **87**, 445 (1948). zitiert aus *C.A.*, **42**, 5552 (1948).
4) W. F. Goebel und G. E. Perlmann, *J. Exp. Med.*, **88**, 479 (1949).
5) P. Desnuelle, S. Antonin und A. Casal, *Bull. soc. chim. biol.*, **29**, 694 (1947).
6) E. F. Jansen, A. L. Curi und A. K. Balls *J. Biol. Chem.*, **189**, 671 (1951).
7) M. Hamada und K. Maekawa, *Sci. Bull. Fac. Agric., Kyusyu Univ.*, **13**, 180 (1951).

gefüllt. Der dabei entstandene Farbenton wurde unter Anwendung von Filter 570 mμ bestimmt. Differenz der Daten für Blindprobe bezeigt Perjodat-Verbrauch. Die dabei gefundenen Ergebnisse sind wie folgende:

Wie man aus der Figur erkennt, ging die Oxydation anfangs bis zur 20 M/M Ovalbumin verhältnismässig schnell vor sich, es bedurfte aber eines Tages, um 30M von Perjodat zu verbrauchen. Diese Ziffer zeigt also, dass die von Perjodat oxydierte Stelle durchschnittlich bei Ovalbumin im Verhältnis von 1 Stelle pro 12~13 Aminosäurereste steht, wenn die Summe der Aminosäurereste in Ovalbumin als 402 angenommen wird[8].

(B). Zur Darstellung von Oxy.-Ovalbumin wurde folgendes Verfahren angewandt. Zu 200 ccm von Ovalbuminlösung (5.5 mg/ccm) wurden je 4.5 ccm (a), 9 ccm (b) und 13.5 ccm (c) von 0.1 N $NaIO_4$ Lösung zugesetzt, und diese Gemische wurden 15 Stunden lang in der Zimmertemperatur (12°) gelassen. Nachdem so gestanden, zeigten die Reaktionsgemische *p*H 6.6~6.8 und enthielten kein Perjodat ausser (c). Hierzu wurden gesättigte Ammoniumsulfatlösungen in folgenden Raten versetzt, um oxydiertes Ovalbumin niederzuschlagen.

(a) 1 ccm Lösung: 2.3 ccm $(NH_4)_2SO_4$—Lösung
(b) „ : 2.0 „
(c) „ : 1.6 „

Jeder Niederschlag wurde in Wasser gelöst und mit Ammoniumsulfat wieder behandelt. Die Umlösung wurde so oft wiederholt, bis Jodverbindung nicht mehr erkennbar war. Die wässerigen Probelösungen wurden zuletzt zu *p*H 5.3~5.5 zubereitet, mit Ammoniumsulfat versetzt, bis sich eine kaum bemerkbare Trübung zeigte, und so gelassen, um die oxydierten Proben zu fällen.

Jede Probe der erhaltenen Oxy.-Ovalbumine ist anscheinend mit Ovalbumin ganz ähnlich.

II. Veränderung in Zusammensetzung infolge der Oxydation. An den oben erwähnten oxyproben wurden Aminosäuren bestimmt, welche erwarten liessen, vermutlich oxydative Zersetzung zu erleiden. So wie es ist, unmittelbar oder nach Hydrolyse mittels Säuren oder Baryta wurde Aminosäure-bestimmung nach verchiedenem Verfahren und mutatis mutandis verrichtet. Die Resultate sind in der Tafel I zusammengestellt.

TABELLE I
VERÄNDERUNG IN ZUSAMMENSETZUNG INFOLGE DER OXYDATION

Probe	a	b	c		
Oxydationsgrad	10 Mol. $NaIO_4$	20 Mol. $NaIO_4$	30 Mol. $NaIO_4$	Oxygelatin	Oxy-Haare
Ausbeute	90 %	83 %	64 %		
$[\alpha]_D^{20}$, (*p*H 7.7)	−55.5°	−55.5°	−83.3°	−118.3°	
Tryptophan	0.2 %	0	0		
Histidin	2.35%	2.33%	2.33%		
HS—	0	0	0		
Arginin	5.5$_0$%	5.3$_4$%	4.8$_1$%	(88%)*	
Tyrosin	3.6$_0$%	3.3$_2$%	3.0$_0$%		
Oxyprolin				(93%)*	
Cystin	1.1$_5$%	1.1$_1$%	1.1$_1$%		Spur (0.05%)

Das Zeichen* zeigt v.H. von zurückbleibenden.

Zur Eiweissbestimmung wandten wir nicht Ninhydrin, sondern Biuret-Reaktion an, weil Oxyprobe mit jenem sich nicht färbte.

Tryptophan wurde nach photokolorimetrischem Verfahren (Filter 530 mμ) bestimmt, unter Anwendung von Farbreaktion, welche aus Tryptophan und Vanillin im Gegenwart von Schwefelsäure entsteht.[9]

Wie sich aus der obigen Tafel erhellt, lässt sich Tryptophan schon in früher Stufe der Reaktion beinahe zersetzen.

Sulfhydryl-Gruppe wurde nach Ferricyanid-Methode bestimmt[10]. Es wurde erkannt, dass die Gruppe-SH auch anfangs angegriffen wurde. Dagegen ist Cystin fast unverändert zurückgeblieben. Ausserdem lässt sich vermuten, dass ein Teil von Cystein durch Perjodat zum Cystin oxydiert wurde. Haare, die zum Nachschlage mit sehr überschüssigem $NaIO_4$ monatelang durchdringend oxydiert wurden, hatten aber nach Hydrolyse nur Spur von Cystin.

Arginin erlitt wenig Veränderung. Dies wurde unmittelbar bestimmt, mit Hilfe der

8) G. R. Tristram, "Advances in Protein Chemistry," Vol, V, s. 137, Academic Press Inc., Publishers, New York, N. Y., (1949).

9) I. Iwamura, H. Hamakawa und Y. Nakahiro, *J. Agric. Chem. Soc. Japan*, **23**, 543 (1947).

10) E. S. G. Barron, "Advances in Enzymol.", Vol. 11, S. 223, Interscience Publishers, Inc., New York, N. Y. (1951).

Sakaguchi'schen Reaktion[11].

Tyrosin litt durch solch eine Oxydation keine erkennbare Veränderung.

Oxyprolin, welches bei Gelatine in Frage steht[12], zeigte keine merkbare Zersetzung.

Zusammenfassung

Ovalbumin wurde durch Perjodat in verschiedenem Grade oxydiert, ohne zu koagulieren.

Oxydiertes Ovalbumin enthielt nicht mehr Cystein und Tryptophan, aber Tyrosin, Histidin und Cystin blieben noch meistenteil zurück.

Aus dem Institut für biologische Chemie der landwirtschaftlichen Hochschule Matsuyama

11) H. T. MacPherson, *Biochem. J.*, **40**, 470 (1946).
12) R. E. Neuman und M. A. Logan, *J. Biol. Chem.*, **184**, 299 (1950).

Immunochemical Studies on Oxidized Ovalbumin

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In the preceding paper it was described that crystalline ovalbumin can, without giving any coagulation, be oxidized in the molar ratio of 10-30 moles of periodate per mole of protein[1]. The oxidized ovalbumin thus obtained was soluble in water at the isoelectric point; accordingly it appears to have been undenatured[3a]. But it is obvious that some groups on protein surface were decomposed by oxidation. Therefore, the present experiment was made with a view to elucidating what rôle those decomposed groups would, as determinant molecular ones, take in connection with the antigenicity of ovalbumin.

Experimental

1. Immune Sera Antisera were prepared by applying ovalbumin and oxi.-ovalbumin (oxidized with 30 moles of periodate) in the following way, after recrystallizing them several times (1). The aqueous protein solution (10 mg./cc.) containing phenol (0.5%) were injected into the auricular vein of rabbits four times at the interval of four days each. The initial dose was 0.5 cc. and each successive one was increased by 0.5 cc., thus the fourth and last one reaching 2 cc. After a week from the last injection, the blood of each rabbit was exsanguinated by cardiocentesia, and each serum was prepared from the blood by the usual method[2].

2. Analytical Methods (a) *Quantitative precipitin reaction.* Quantitative analyses in anti-ovalbumin sera and anti-oxi.-ovalbumin system were carried out as follows. Accurately measured solutions of antigens and sera were mixed and allowed to stand at 37° for 3 hours[3b]. The precipitates were centrifuged at room temperature and washed twice with 2 cc. of 1% aq. NaCl solution at 0° and centrifuged again.

(b) *Determination of nitrogen in the specific precipitates.* Nitrogen in the precipitates was determined as following. After 1 cc. conc. H_2SO_4 and 20 mg. K_2SO_4 (without addition of $CuSO_4$) were poured directly in the conical centrifuge tube containing precipitates, decomposition was conducted by heating for one hour. On the digested solution, ammonia was determined by the nesslerization according to modified Thompson and Morrison method[4]. Namely, the solution was poured into a 50 cc. measuring flask, neutralized completely with NaOH, and then filled up with water to the mark. To 20 cc. of this solution, were added 0.2 cc. of 50% Rochelle salt solution and 0.2 cc. of Nessler solution. After the solution was allowed to stand at 20° for 20 min., the transmittancy of light was measured by a photoelectric colorimeter using filter S 44. Previous adjustment is necessary to keep the ammonia content within a range of 1.7—3.5 γ/cc. When the solution becomes turbid, after adding Nessler solution and warming, this sample is too conc. as to ammonia and therefore must be further diluted to repeat the same process as mentioned above.

(c) *Assay of supernatant solutions.* After precipitates of antigen-antibody complex were centrifuged, the supernatant solutions were tested qualitatively for both excess antigen and antibody. Approximately one-third of the solution was assayed for excess antigen and the rest for excess antibody by the addition of small amounts of antiserum and antigen respectively[5,6].

1) The foregoing report; reported in the meeting of the *Agr. Chem. Soc. Japan.* (at Kyoto, May 1951).
2) J. A. Kolmer and F. Boerner, "Approved Laboratory Technic." (New York) 1945, p. 616.
3a) P. H. Maurer and M. Heidelberger, *J. Am. Chem. Soc.*, **73**, 2070 (1951).
3b) P. H. Maurer and M. Heidelberger, *J. Am. Chem. Soc.*, **73**, 2076 (1951).
4) J. F. Thompson and G. R. Morrison, *Anal. Chem.*, **23**, 1153 (1951).
5) L. R. Wetter and H. F. Deutsch, *J. Biol. Chem.*, **192**, 237 (1951).
6) H. F. Deutsch, *J. Biol. Chem.*, **185**, 377 (1950).

TABLE I

ADDITION OF INCREASING AMOUNTS OF OVALB. AND OXI.-OVALB. TO 1.0 cc. OF ANTI-OVALBUMIN SERUM

Kind of antigen	Antigen N added, mg.	Total N pptd., mg.	Antibody N by diff., mg.	Antibody N / Antigen N	Supernatant tests
Ovalbumin	1.5760	0.0752			Excess antigen
	0.1576	0.5024	0.3448	2.2	,,
	0.0628	0.6531	0.5903	9.4	Excess antibody
	0.0314	0.4710	0.4396	14.0	,,
	0.0157	0.3014	0.2857	18.2	,,
	0.0063	0.1342	0.1279	20.3	,,
	0.0031	0.0688	0.0657	21.2	,,
Oxi. ovalbumin	1.5760	0.0837			Excess antigen
	0.1576	0.5495	0.3919	2.5	,,
	0.0628	0.6154	0.5526	8.8	Excess antibody
	0.0314	0.4145	0.3831	12.2	,,
	0.0157	0.2434	0.2277	14.5	,,
	0.0063	0.1071	0.1008	16.0	,,
	0.0031	0.0539	0.0508	16.4	,,

TABLE II

ADDITION OF INCREASING AMOUNTS OF OVALBUMIN AND OXI.-OVALBUMIN TO 1.0 cc. OF ANTI-OXI.-OVALBUMIN SERUM

Kind of antigen	Antigen N added, mg.	Total N pptd., mg.	Antibody N by diff., mg.	Antibody N / Antigen N	Supernatant tests
Ovalbumin	0.1576	0.0523			Excess antigen
	0.0314	0.2449	0.2135	6.8	,,
	0.0157	0.1649	0.1492	9.5	Excess antibody
	0.0063	0.0775	0.0712	11.3	,,
	0.0031	0.0400	0.0369	11.9	,,
Oxi. ovalbumin	0.1576	0.0542			Excess antigen
	0.0314	0.2355	0.2041	6.5	,,
	0.0157	0.1696	0.1539	9.8	Excess antibody
	0.0063	0.0832	0.0769	12.2	,,
	0.0031	0.0434	0.0403	13.0	,,

Results and Discussion

The results are shown in Tables I and II. It is apparent that there is no difference between the homogeneous reaction and the cross one in precipitin reaction upon ovalbumin-serum, except in the case of the less antigen region.

The slight difference which was found in the less antigen region, suggests that there is some loss on antigen surface which should be combined complementarily. On the other hand, there is also no difference between homogeneous and cross reaction in the reaction upon oxi.-ovalbumin-serum, except in the case of the less antigen region. In the less antigen region, the precipitate is formed somewhat more in the case of homogeneous reaction. The relation between the ratio of the antibody N to antigen N in the precipitates and the amount of antigen N increasingly added are shown in the Figure 1. As

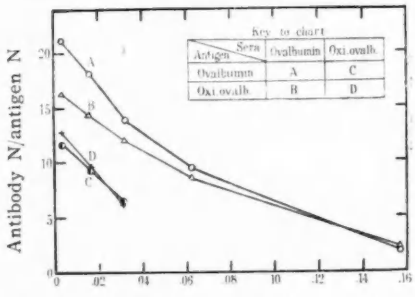

Fig 1. Relation between the ratio of antibody N to antigen N in the precipitates and antigen N added to the serum dilution.

shown in the figure, it would seem that the oxi.-ovalbumin is not serologically heterogeneous, since the curve concerning serum of oxi.-ovalbumin is in straight line[6].

From the comparison between Tables I and II, it is clear that serological specificity was diminished considerably by the oxidation. Heidelberger and his collaborator[3b] reported that the deaminated proteins, even though one third of free NH_2-group was eliminated off, retain their serological specificity, so long as protein was not denatured.

The oxi.-ovalbumin reported here can be regarded as not denatured[7a]. Therefore, this difference between those facts is to be ascribed to the difference in the contribution to serological specificity between end-NH_2-group[7] and those groups on protein surface which were oxidized by periodate. In other words it seems that the specificity is more attributable to tryptophan, hydroxylamino acids and tyrosine, situated on protein surface, than to end NH_2-group.

7) R. R. Porter and F. Sanger, *Biochem. J.*, **42**, 287 (1948).

The decrease in serological specificity of oxi.-protein probably is not due to the fact that the surface, as in case of denatured protein, is random and has no definite folding, but to the fact that some determinant groups on the surface is decomposed by oxidation.

Summary

Using the oxidized ovalbumin, quantitative precipitin reactions were brought forward. There was some difference between the homogeneous reaction and the cross one in the precipitin reaction upon ovalbumin- and oxidized ovalbumin-serum only in the case of the less antigen region. The serological specificity of ovalbumin diminished considerably by the periodate-oxidation.

The authors wish to express their gratitude to Mr. M. Kushibe for his valuable assistance in this investigation.

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Studies on Some Physico-Chemical Properties of Ion-Exchange Resin. I. Heat of Wetting

By Tatsuo MATSUURA

(Received February 20, 1954)

Introduction

Recently, as an ion-exchange resin has been of great importance in its application in the field of chemistry and chemical engineering, it has now come to be noticed also by many physical chemists as a typical sample of an insoluble high-polymeric electrolyte. Because of its having both a high-polymeric and electrolytic nature, ion-exchange resin seems to have many characteristic properties, one of which is, unlike other high-polymers, the rapid and reproducible swelling in water, which is seen remarkably at the low cross-linked resin, while it is little seen at the high cross-linked resin. In both cases, if the sample has been dried enough, considerable heat is evolved at the moment when it is immersed in water. The author, in this paper, attempted to investigate this phenomenon as a first approach to the fundamental nature of ion-exchange resin. The measurements were made on various forms and states of some cation and anion-exchange resins, and it was found that the amounts of heat evolved depended largely on their forms in such a way that the sequence with the latter was almost parallel with the series of the heats of hydration of the ions. Theoretical considerations were also attempted with the adoption of the appropriate values for the heats of hydration of ions and some assumptions on the dry state of resion.

Experimental Procedure

Samples Used. Samples used are classified into three types; that is, the strongly acidic cation-exchange resins, the weakly acidic cation-exchange resin, and the strongly basic anion-exchange resins.

The first are polystyrene-divinylbenzene sulfonic acid exchangers and are also classified into the following three types according to their cross-linkage. The lowest cross-linked one is easily made from polystyrene by sulfonation with

chlorsulfonic acid. The other two are commercial, more or less highly cross-linked, "Amberlite IR-120" and "Amberlite IR-112," whose cross-linkage is said to be 8% to 10% and about 5% divinylbenzene (DVB), respectively.

As a sample of the weakly acidic resin, carboxylic "Amberlite IRC-50" was used. It is a copolymer of methacrylic acid and about 10% DVB.

Anion-exchange resins used, commercial "Amberlite IRA-400" and "Amberlite IRA-410," are quarternary ammonium salts with the network structures of polystyrene-divinylbenzene.

About 1 g. to 3 g. of resin was used for each run. Well-conditioned, uniform-sized (about 100 Meshes) resin was taken in a column, through which the solutions containing the concerning ion of about ten times as much as the exchange capacity of the resin bed were passed; then it was washed by distilled water, except in the cases described below, till the effluent was freed from the regenerant. In the case of the salt form of carboxylic resin, the washing was made by dilute alkali of about 0.001 N, lest the adsorbed ions might be hydrolyzed[1]. This care was also given in the case of anion-exchange resin, where the exchange was more easily obtained at a lower *p*H range. The drying of samples was done first in an air-oven, then in a desiccator, finally in a test tube with a glass joint stopper in an Abderhalden's dryer at the boiling temperatures of alcohols or water under the reduced pressure of about 0.02 mm Hg. It took from a few days to sometimes about twenty days for the samples to have the constant weights within 1 mg.

The partially desulfonated resins were prepared as follows: the synthesized resin was boiled for about one week with the mixture of conc. sulfuric acid and an almost equal volume of water, at about 150°C. The sample was blackened by this treatment, but the decrease in the exchange capacity was not so great as expected, showing the stability of this sort of resin.

The measurements on the partially wetted samples were made only on acid and sodium forms of the strongly acidic resins, which were prepared either by partial desiccating of resin or by moistening the completely dried resin with damp air. The contents of water in the samples were easily determined by titrating the exchange capacities of resins by column or batch method, and the values obtained agreed with those by the change of weight with drying or wetting.

Calorimetric Measurement. Fig. 1 shows the experimental apparatus. When the temperature of the calorimeter reached about 18°C, the readings of the Beckmann thermometer were begun at intervals of 30 seconds with the constant stirring by the stirrer S. Then the samples was carefuly and rapidly thrown from the test tube reserved at the thermostat into the water through the hole C of the vessel. The temperature rise caused by the wetting of the resin occurred immediately

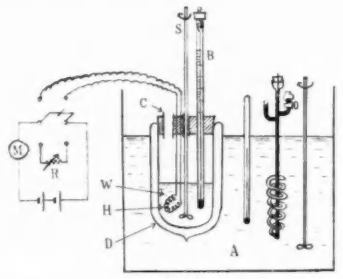

Fig. 1

A: thermostat. B: Beckman thermometer. D: Dewar vessel. S: a plastic stirrer. C: a hole. W: about 180 cc. water. H: a heater. R: resistance box almost equalled with the resistance of the heater. M: a calibrated ammeter (1 amp. range).

after that, sometimes with a noise of its decrepitation. Soon after the temperature-time diagram became flat, another temperature rise which was caused by introducing an electric current through the heater H for some seconds was added to determine the whole heat capacity. This state of matters was shown in a diagram such as Fig. 2.

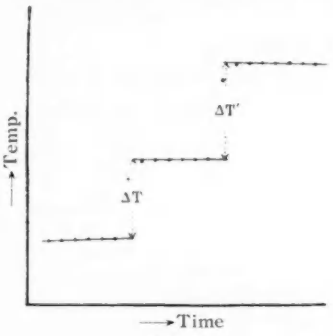

Fig. 2

in which these temperature changes were both in the order of 0.1°C to 1.5°C. By determining the weight of samples, the value of the heats of wetting was expressed in cal. per gram, as well as in kcal. per equivalent after the measurement of exchange capacity.

Exchange Capacity. Ordinarily after the calorimetric measurements were done by the above mentioned procedure, the exchange capacity of the resin, i.e., the number of exchange sites per unit, was measured by the column method. In the strongly acidic resins, each salt form was reduced to hydrogen form by the use of 4 N hydrochloric acid as regenerant and was completely washed by distilled water, then by passing the sufficient amount of neutral sodium chloride solution the hydrogen ion that was driven into the effluent was titrated by a standard alkali. In the case of weakly acidic resins, the measure-

1) M. Honda, *J. Chem. Soc. Japan* (Pure Chem. Sect.), **71**, 183, 440 (1950), *Japan Analyst*, **1**, 120 (1952).

ments were made by column or batch method, where alkaline, instead of neutral, solution was used to obtain a complete exchange and the washing was also made by dilute alkali.

At the anion exchange resins, the reduced state was selected at the chloride form, and the titration was performed by Mohr's method.

Results and Discussion

The results obtained are shown in Table I and Fig. 3. From these data it is seen at once as follows: that the values of the heat of wetting of ion exchange resin in general are considerably great as compared with the other high polymers such as starch (about 30 cal./g.)[2], and that there is a remarkable difference between them according to their forms. The sequence at the strongly acidic resin, that is, the fact that the more the valency of the ion or the higher the positon of the ion in the group, the greater is the value of the heat, coincide with the lyotropic series of ion, which undoubtedly has an important relation to the hydration of ions. The degree of difficulty in the dehydration of resin, namely, the period required for the resin to be completely dried in Abderhalden's dryer, was also consistent with this series. Thus the aluminium or magnesium form of this sort of resin was found to have the greatest heat evolution, which was contrary to the result mentioned in a review[3].

As the cation exchange resins were comparatively stable, even the treatment at 100°C did not seem to bring any serious effects, except only that some change of color (getting brown) took place at the hydrogen form, but without any change of exchange capacity. The anion exchange resins were so unstable that they decomposed more or less with a peculiar smell at comparatively low temperatures, especially at the hydroxyl ion form, which lost almost all its capacity by the drying at 65°C.

The results of the weakly acidic type resin were very interesting from the following respects. That is, the largest heat evolution per unit weight was seen with this sort of resin, and the position of the hydrogen form was much lower than that of the other forms, compared with the results of strongly acidic resins. The latter fact may be due to the undissociative nature of the hydrogen atom of the weakly acidic carboxylic group, and the value suggests the magnitude of the hydrogen bond between this hydrophylic radical and the water molecules.

In comparison with the great dependence on the variety of adsorbed ions, the heats of wetting seem scarcely to depend on the cross-linkage as far as this experiment is concerned. Only in the case of the hydrogen form of sulfonic acid type resin an effect just like this was noticed above the experimental error, that is, there was some difference depending on the cross-linkage. However, this probably is accounted for by the considerably high value of heat of dilution of hydrogen ion, and not by the difference of degree of swelling. Thus, such a rather rough measurement as this could not pursue "the swelling heat", which, even if it really exists, must be very small, because the swelling is naturally a phenomenon greatly related rather to the entropy change than to the enthalpy change.

The attempt to find any dependence on the exchange capacity led to the measurement on the desulfonated resin, where although the decrease in the exchange capacity by this method was attained to some extent, the accompanying carbonization of the resin seemed to make the matter complicated.

The calculated values of exchange capacity in a salt form of cation exchangers were derived from the standard hydrogen form by the following formula:

$$E_{MR}=\frac{E_{HR}}{1+E_{HR}\,(m/z-1)}\ (m\text{ eq. per g.}) \quad (1)$$

where E_{MR} is exchange capacity of the concerned salt form (MR), E_{HR} of acid form (HR), and m and z are molecular weight and valency of the adsorbed ion, respectively.

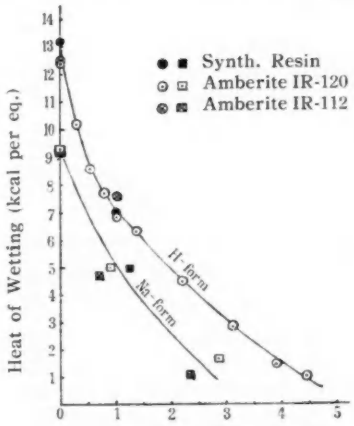

Fig. 3

2) T. Nakagawa, This Bulletin, **26**, 169 (1953).
3) G. E. Boyd, *Ann. Rev. of Phys. Chem.* **2**, 306 (1951).

TABLE I

THE HEAT OF WETTING OF ION EXCHANGE RESIN

Species of resin	Adsorbed ion form	Drying data Temperature	period	Heat of wetting		Exchange capacity obs.	calc.	Apparent wet swollen volume in water
		(°C)	(days)	(cal./g.)	(kcal./eq.)		(meq/g.)	cc./g.
Synthetic		(Strongly Acidic Cation Exchange Resin)						
Resin	Al	100	20	83.8	19.3	4.15	4.34	
"	Mg	100	20	82.5	19.7	4.20	4.28	
"	H	100	5	58.7	13.1	4.49	(4.50)	10
"	H	78	7	59.4	13.2	4.50	(4.50)	
"	Ca	100	20	51.0	12.3	4.02	4.15	
"	Li	78	5	45.3	10.3	4.24	4.38	
"	Ba	78	10	35.8	10.4	3.29	3.44	
"	Na	78	5	37.2	9.1	4.08	4.09	
"	K	78	4	18.1	4.8	3.77	3.84	
"	NH_4	78	3	17.6	4.2		4.18	
"	Rb	78	5	13.5	4.3	3.12	3.26	
Amberlite								
IR-120	H	78	5	55.9	12.4	4.50	(4.50)	2.3
"	Na	78	5	37.8	9.3	4.08	4.09	
"	K	78	5	22.7	5.9	3.84	3.84	
Amberlite								
IR-112	H	78	4	56.3	12.5	4.48	(4.50)	5.4
"	Na	78	3	37.7	9.2	4.16	4.09	
"	K	78	3	18.3	4.8	3.75	3.84	
Desulfonated	H	78	5	55.6	14.6	3.81	(3.81)	8.5
Resin I	Na	78	5	37.8	10.6	3.56	3.51	
Desulfonated	H	100	5	60.5	14.1	4.30	(4.30)	9.0
Resin II	Na	100	5	34.9	8.7	3.94	3.94	
Amberlite		(Weakly Acidic Cation Exchange Resin)						
IRC-50	Ca	78	20	122		4.8	7.9	
"	Na	78	8	113.5	15.3	7.4	7.7	
"	Na : H=2 : 3	78	6	61.6	7.6	8.1	8.6	
"	H	100	8	30.7	3.2	9.6	(9.35)	5
"	H	78	3	30.4	3.3	9.1	(9.35)	
Amberlite		(Strongly Basic Anion Exchange Resin)						
IRA-410	Cl	78	4	17.0	4.6	2.8		
"	NO_3	65	5	7.3	2.5	2.9		
Amberlite								
IRA-400	SO_4	56	20	41.8	14	3.0		
"	CH_3COO	56	4	37.4	11.4	3.3		
"	I	56	3	12.9	4.6	2.8		

*Parentheses show the standard values selected for calculating the exchange capacity of other forms by Eq. 1.

The observed and calculated values of the exchange capacity were as a whole in good agreement, except the tendency that the former were a little less than the latter. This may be accounted for as follows. (1). Incomplete drying or impurity in reagents. (2). Incomplete regeneration may be probable at the measurement of exchange capacity for the ions which have higher selectivity than hydrogen. (3). For polyvalent ion forms, the occurrence of basic ion such as $Mg(OH)^+$ may increase the apparent equivalent of the ion.

The measurement of the partially wetted samples also gave a very interesting result. As is shown in Fig. 3, at the hydrogen form of strongly acidic resins, about five water molecules per one exchange radical were found to be involved in the heat of wetting, the greatest part of which could be attributed

to the hydration of the hydrogen ion. The inflection observed on the curve at the point of one water molecule per one exchange radical may be a proof of the strong attraction between the bare hydrogen ion and one water molecule, forming the hydronium ion (H_3O^+). Considering the fact of little dependence on the cross-linkage, this number, about five water molecules per one hydrogen ion, might be said to be the hydration number of the hydrogen ion. The corresponding number for the sodium ion was found to be about three. The author believes that this can be one of the methods to estimate the hydration number of ions. On the other hand, from the wet swollen volume per gram of strongly acidic resin, the resin phase may be regarded as a concentrated solution of about 5 N for IR-120 and 0.5N for synthetic resin. These correspond to the fact that there exist from about ten to one hundred water molecules per one exchange radical, and we may say that the above mentioned five molecules are the bound water and the other are the interstitial water of hydrogen form of this resin.

The following are theoretical considerations on the heat of wetting of sulfonic acid type cation exchanger. The heat of wetting is regarded to be the negative value of the enthalpy change in wetting, ΔH_{WET}, which is the difference of heat content when the completely dried state of resin is brought to the wetted state. Now, as the model which is shown in Fig. 4, this process may be

Fig. 4

divided hypothetically into the four steps. (1). The original dried sample is dissociated into fragments in a vacuum, so as for each ion pair as well as the links between structurally bound negative radicals to be separated infinitely. The enthalpy change at this stage is the sum of ΔH_D, due to the dissociation of ion pairs, and ΔH_B, which concerned with the energy required to break the bonds of the polymer network. (2). Then the completely dissociated ions (both cation and radical anion) are brought from the vacuum into the water, when the hydration of ions takes place. The enthalpy change here is caused by the hydration of both ions, $\Delta H_{HYD.}$ (3). Thirdly, the wetted yet infinitely separated ions are concentrated as far as the concentration which corresponds to the swelling state of resin. The accompanying enthalpy change is the negative value of that of dilution from the concentration to the infinite dilution, $\Delta H_{DIL.}$ (4.) Finally, while the concentration of ions are reserved constant on the other hand, the recombination into the swollen resin state takes place. Then the enthalpy change involved is only one term, which is required to combine the separated polymer bonds, thus giving $-\Delta H_B$, just the negative value of the term mentioned at the first step. After all, the change in heat content by wetting is expressed as follows:

$$\Delta H_{WET}=\Delta H_D+\Delta H_{HYD}-\Delta H_{DIL.} \quad ...(2)$$

in which $\Delta H_{DIL.}$, something similar to the heats of dilution of a few normal solution of usual salts or acids, being only 1 or 2 kcal. per mol at the most, can be neglected at the first approximation, as is compared with the order of about 100 kcal. or much more of $\Delta H_{HYD.}$ Then,

$$\Delta H_{WET}=\Delta H_D+\Delta H_{HYD.} \quad .....(3)$$

As for the energy of hydration of ions, although we have several studies in connection with the ionic radii or hydration numbers, it seems that a complete interpretation with relation to the actual hydrated radii of ions has not yet been obtained, but that only semi-empirical formula originated by Born[4] are still now prevailing[5,6]. The author, too, chose the following formula presented by Latimer[5] as the values of $\Delta H_{HYD.}$

$$\Delta H_{HYD}=\frac{-Nz^2e^2}{2(r_i+b)}\left(1-\frac{1}{D}-\frac{T}{D^2}\frac{dD}{dT}\right)$$

c.g.s.e.s.u. per mole, ...(4)

where N is Avogadro's number,
e, charge of electron,
z, valency of ion,
r_i, crystal ionic radius of ion,
b, correction value,
D, dielectric constant of medium, and
T, absolute temperature.

Inserting known figures at room temperature into Eq. 4, one obtains,

4) M. Born, *Z.f. Physik.* **1**, 45 (1920).
5) W. M. Latimer, K. S. Pitzer, and C. M. Slansky, *J. Chem. Phys.*, **7**, 108 (1939).
6) K. B. Yatsimirskii, *J. Gen. Chem.* (U. S. S. R.), **17**, 2019 (1947), *C. A.* **42**, 4909, (1948).

$$\Delta H_{HYD} = -\frac{165\,z^2}{r_i+b} \text{ kcal. per mol.} \quad ...(5)$$

where r_i is expressed in Å. The numerical constants b, which have a serious effect on ΔH_{HYD}, were chosen 0.85 for monovalent cations except hydrogen ion, as was done by Latimer[5]. As he did not give the value for hydrogen, ΔH_{HYD} for this ion was taken from the Fajans' value, 255 kcal. per mol.[7]. b's were chosen 0.8 for divalent and trivalent cations and 0.3 for anions, respectively, followed by Yatsimirskii[5]. Thus the ΔH_{HYD} for cation is shown in the third column in Table II. The value of ΔH_{HYD} for sulfonic acid anion with polymer fragments was arbitrarily assumed to be 40 kcal. per eq., which corresponds to an ionic radius of 3.82 Å according to Eq. 5. As the following discussion refers to, the "true ionic radius of sulfonic acid group" may be assumed to have the value, 1.40 Å, for which, according to Eq. 5, ΔH_{HYD} is calculated to be 97 kcal. per Eq. The discrepancy in these values might be interpreted as follows: the attachment of the extra polymer chain may enclose the sulfonic radical, resulting the increase in effective radius; or the hydration of the sulfonic acid group is hindered sterically by the polymer network. The fourth column in Table II is the sum of ΔH_{HYD} for cation and 40 kcal.

TABLE II

THEORETICAL CONSIDERATION ON THE HEAT OF WETTING OF SULFONIC ACID TYPE CATION EXCHANGE RESIN

Ion Species	Crystallographic Ionic Radii* Å	ΔH_{HYD} for cation kcal./eq.	ΔH_{HYD} total "	ΔU "	ΔH_D calc. "	ΔH_{WET} calc. "	ΔH_{WET} obs. "
H	(0)	−255	−295		335	+40	−13.1
Li	0.60	−121.2	−161	166	149	−12	−10.3
Na	0.95	− 94.6	−135	141	127	− 8	− 9.2
K	1.33	− 75.8	−116	122	110	− 6	− 4.8
Rb	1.48	− 69.2	−109	115	104	− 5	− 4.3
Mg	0.65	−228	−268	283	255	−13	−19.7
Ca	0.99	−189	−229	243	218	−11	−12.3
Ba	1.35	−154	−194	211	190	− 4	−10.4
Al	0.50	−382	−422	425	383	−39	−19.3

* By Pauling

In order to deduce the calculated values for ΔH wet by Eq. 3, it is necessary to calculate the appropirate values for ΔH_D. For this purpose, the following assumptions were made. (1). The negative charge on a sulfonic acid group exists on the position of the oxygen atom which is the immediate neighbor of the adsorbed cation, thus as the "ionic radius of sulfonic acid group," the van der Waals radius or the ionic radius of oxygen, which both happened to have the same value, 1.40 Å[8], was taken. (2.). The dry state of resin except hydrogen form is assumed to be similar to an ionic crystal, that is, the adsorbed ion and radical anion are coupled as ions also at the dry state, but because the pairs are distributed amorphously, the electrostatic interaction is almost limited to the coupled ions, thus the Madelung constant can be assumed to be unity. (3). Thus the electrostatic attraction by the Coulomb force of ion pairs can be expressed by

$$\Delta U = \frac{Ne^2\,z'}{(r_i+1.40)} \times 10^8$$

c.g.s. e.s.u. per Eq.,.....(6)

where N, e, and r_i are as described above; z', a corrected valency of ion considering the repulsion of the ions of same charge, is 1 for monovalent adsorbed ions, 1.75 for divalent ions, and it depends on ionic radius for trivalent ion (2.44 for aluminium). (4). ΔH_D, the energy required to separate the ion pairs infinitely, so to speak the "crystal energy of ion-exchange resin," is estimated roughly as much as about ninety percent of the electrostatic attraction ΔU, considering the existence of the repulsion term. This figure was taken from the average value of several species of mono-monovalent ionic crystal[9]. (5). For

7) K. Fajans, *Naturwissenschaften*, **9**, 729 (1921).
8) L. Pauling, "The Nature of the Chemical Bond," p. 189, 346 (1939).
9) E. A. Moelwyn-Hughs, "Physical Chemistry," p. 599, (1940).

hydrogen form, we adopt a different model; that is, the atoms in the dry state of that form are no longer ionized but exist as covalent bond, and

$$\Delta H_D = D + I - E, \qquad \text{.....(7)}$$

where D is energy required to separate the covalent bond between the hydrogen atom and residual sulfonic radical, and $I-E$ is the difference between the ionization energy of hydrogen atom and the electron affinity of the sulfonic radical. As the value for D, 110 kcal. per mole, the covalent bond energy of O–H bond in water molecule, was conventionally used. The value for I is 313 cal.; and for E, 88 kcal., the value of electron affinity of oxygen atom, was conventionally used.

Thus the calculated ΔH_{WET} is shown in column 7, Table II. The fair agreement between ΔH_{WET} observed and calculated, considering the fact that it is derived as the difference of the two large values, suggests that these assumptions just made are not going too far except in some cases. At the hydrogen form, where the deviation is very large, it is necessary to use more refined values for D and E. For divalent and trivalent cation forms, both the uncertainty in ΔH_{HYD} and the probable deviation from the above mentioned factor (90%) are enough to explain the poor coincidence between theory and experiment.

Summary and acknowledgment

(1) The measurement of heats of wetting of strongly acidic and weakly acidic cation exchange resins and strongly basic anion exchange resins was made by a simple method and their great dependence on the adsorbed ion species was found.

(2) The series of heat of wetting with ion forms is almost parallel with the lyotropic series of ions, thus it has been shown that this penomenon has an important relation to the hydration of the ions. The acid form of the weakly acidic resin occupies a special position, and this is attributed to the undissociative nature of carboxylic radical.

(3) The result on the partially wetted samples supports the view that only a few water molecules which may be called "bound water" take part in the heat of wetting or hydration of ions.

(4) A theoretical interpretation based on a few assumptions was attempted and a fair agreement with the experimental results was obtained.

The author wishes to express his hearty thanks to Dr. Masatake Honda of the Department of Chemistry of the Faculty of Science in the University of Tokyo, for his kind guidance and encouragement extended to the author while he was with him at Rikkyo University.

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The Molecular Structure of cis-1,2-*Dichlorocyclohexane*

By Kunio KOZIMA, Kiyoshi SAKASHITA and Yoshiyuki TAKEISHI

(Received March 12, 1954)

1,2-Disubstituted cyclohexanes have two geometrical isomers. One is called the *cis*-isomer and the other the *trans*-isomer. By use of the usual symbols the molecular structure of the former is represented by (1e, 2p) or (1p, 2e). As for 1,2-dihalocyclohexanes the *cis*-isomers were not obtained for a long time. However, a new 1,2-dichlorocyclohexane, presumably the *cis*-isomer, has recently been described by Carrol et al.[1]

If the compound is really *cis*-1,2-dichloro derivative, it should have the following two properties, since its cyclohexane ring should take the chair-form as in the case of the similar derivatives[2-4] already reported: (1) The compound has not the "inverted isomer".[2-4] (2) The value of dipole moment is nearly the same as that determined by us for the (1e, 2e) form of *trans*-1,2-dichloro derivative[3], namely 3.1 D.

The main purposes of this research are to ascertain whether or not the compound is the *cis*-isomer by measuring the vibration

1) B. Carrol, D. G. Kubler, H. W. Davis and A. M. Whaley, *J. Am. Chem. Soc.*, **73**, 5382 (1951).

2) K. Kozima and T. Yoshino, ibid., **75**, 166 (1953).

3) K. Kozima, K. Sakashita and S. Maeda, ibid., **76**, 1965 (1954).

4) K. Kozima, *Bull. Tokyo Inst. Tech.*, **1952**, 1. (in Japanese).

spectra and the dipole moment and, if so, to know the properties of the compound.

Experimental

Preparation and Purification of the Compound.—According to the method of Carrol et al.[1] we prepared the 1,2-dichlorocyclohexane by reacting *trans*-1,2-cyclohexene chlorohydrin with thionyl chloride in the presence of pyridine. The product was purified by the fractional distillation in vacuo. The boiling point is 71.0~71.2° at 8.5 mm.

Electric Dipole Moment.—For the measurement of dielectric constants the same instrument was used as before. The data are listed in Table I, where f denotes the mole fraction and P_2 the molar polarization of the solute. The measured values of P_2 are in various cases almost independent of the low concentrations which were used. Therefore, the mean value of P_2 was taken as $P_{2\infty}$ in all cases. The dipole moment was calculated as $\mu=0.0128\sqrt{(P_{2\infty}-R_D)\cdot T}$ using the molar refraction R_D (37.5 cc) calculated from the atomic refractions for the D-line.

TABLE I

DIPOLE MOMENTS

in benzene

$t=30°$, $P_{2\infty}=231$, $\mu=3.10$ D.

f	ε	d	P_2
0.0000	2.2625	0.8675	
0.007093	2.3562	0.8709	230
0.008311	2.3738	0.8715	232

in heptane

$t=30°$, $P_{2\infty}=237.5$, $\mu=3.15$ D.

f	ε	d	P_2
0.0000	1.9056	0.6753	
0.01328	2.0026	0.6814	237
0.01492	2.0157	0.6823	238

in heptane

$t=45°$, $P_{2\infty}=225.5$, $\mu=3.13$ D.

f	ε	d	P_2
0.0000	1.8829	0.6625	
0.01328	1.9776	0.6687	225
0.01492	1.9837	0.6696	226

in carbon tetrachloride

$t=40°$, $P_{2\infty}=227$, $\mu=3.12$ D.

f	ε	d	P_2
0.0000	2.1928	1.5563	
0.006699	2.2690	1.5526	227
0.007255	2.2746	1.5519	227

Vibration Spectra.—For Raman spectra the same spectrograph was used as in our earlier work. The spectra of the compound were observed for the solid and liquid states. Regarding infrared spectra measurements were made with Perkin-Elmer spectrometer Model 112 equipped with NaCl and KBr Prisms. The results of the wave number readings for both spectra are shown in Table II.

TABLE II

VIBRATION SPECTRA

Raman* (solid)	Raman* (liquid)	infrared** (liquid)
cm^{-1}	124 (4) cm^{-1}	cm^{-1}
193 (1)	193 (3)	
278 (2)	278 (4)	
348 (10)	346 (10)	
365 (1)	367 (2)	
?	474 (1)	473 (vw)
510 (5)	510 (8)	511 (m)
535 (10)	541 (10)	543 (s)
681 (10)	695 (10)	695 (vs)
735 (9)	741 (9)	741 (vs)
816 (3)	818 (4)	816 (s)
833 (3)	833 (5)	834 (s)
		~845 (vw)sh
876 (2)	876 (4)	877 (vs)
902 (1)	902 (3)	906 (m)
985 (7)	987 (8)	986 (vs)
		~1008 (vw)sh
1026 (2)	1026 (4)	1025 (vw)
1057 (2)	1057 (3)	1057 (w)
1079 (2)	1079 (2br)	1078 (w)
		1085 (w)
?	1133 (1)	1131 (w)
1191 (1/2)	1191 (1)	1191 (m)
1220 (1/2)	1218 (0)	1217 (w)
	1226 (2)	1226 (m)
		~1262 (vw)sh
1267 (3br)	1267 (5br)	1270 (s)
1296 (1/2)	1296 (1)	1298 (m)
?	1314 (1)	1313 (w)
	1335 (1)	1336 (w)
1346 (1br)		~1346 (w)sh
	1355 (1)	1356 (m)
1442 (4br)	1435 (3)	1446 (vs)
	1443 (6)	
		2169 (w)
		2695 (vw)
	2847 (2)	2880 (m)
2903 (3)	2904 (4)	
2920 (3)	2920 (4)	
2941 (4)	2938	2937 (vs)br
2950 (4)	2955 (6br)	
2982 (2)	2977	

* As for the Raman spectra the numbers shown in the brackets are the visually estimated relative intensities.

** The infrared spectra were not investigated below 430 $cm.^{-1}$ The letters shown in the brackets mean the relative intensities. v=very, s=strong, m=medium and w=weak. sh=shoulder.

Discussion

It is seen from Table I that the dipole moment of the compound does not appreciably vary with temperature or solvent. From Table II the following facts may be clear:

(1) The number of the Raman bands and their relative intensities do not vary except for the disappearance of the several very weak lines of the liquid spectrum when the measuring state is changed from liquid to solid. (2) The infrared and Raman spectra agree with each other perfectly regarding the number of the bands and approximately regarding the relative intensities except the very faint bands and the region above 2000 cm^{-1}.

Two possibilities for interpreting the facts mentioned above come out: (1) The molecule does not possess the inverted isomer; or (2) The molecule has the inverted isomer, but the energy difference between the isomers is so great that only one of the two forms can exist. A case in which the energy difference between the isomers is nearly zero or small can be left out of consideration, because of the number of the bands (for instance, the number of the bands assigned to the C—Cl stretching vibrations is two, as might be naturally expected from the formula of $C_6H_{10}Cl_2$).

It is not immediately clear that the dichlorocyclohexane prepared from 1,2-cyclohexene chlorohydrin, as described above, is 1,2-dichlorocyclohexane. Taking this point into consideration these two possibilities will be discussed.

Each of the three position isomers of dichlorocyclohexanes has two geometrical isomers—the *cis*- and *trans*-isomers. Of these six isomers only three have inverted isomers; viz. *trans*-1,2-, *trans*-1,4- and *cis*-1,3-dichlorocyclohexanes. Regarding the first two of them we have already reported[2,3]. Then, only *cis*-1,3-isomer remains to be considered. The structures of *cis*-1,3-dichloro derivative are represented as follows:

$$(1p, 3p) \rightleftarrows (1e, 3e).$$

The distance between the two chlorine atoms of the (1p, 3p) structure is calculated to be about 2.5 Å, assuming the usual values for the C—C and C—Cl nuclear separations and the tetrahedral valency angle for the carbon atoms of the cyclohexane ring which has the "chair" form. Comparing this distance with twice the van der Waals' radius of the chlorine atom, we can expect that the (1p, 3p) structure will be unstable as compared with the (1e, 3e) structure so that only the latter can exist. Therefore, it is not immediately clear that the compound in question is not *cis*-1,3-dichloro derivative. However, upon the basis of simple vector addition of bond moments, it is calculated that the moment of the (1e, 3e) structure of *cis*-1,3-dichloro derivative, $\mu_{1,3}$, and that of *cis*-1,2-dichloro derivative, $\mu_{1,2}$, are represented, respectively, as follows:

$$\mu_{1,3} = 2\mu_0 \cos \theta/2$$
$$\mu_{1,2} = 2\mu_0 \sin \theta/2$$

where μ_0 is the sum of the moment of C—Cl bond and that of C—H bond, and θ is the tetrahedral valency angle of carbon atom. Estimating the value of μ_0 at 1.80 or 1.90 D, we can calculate the values of $\mu_{1,3}$ and $\mu_{1,2}$ obtaining the following results;

$$\mu_{1,3} = 2.08 \text{ or } 2.19 \text{ D}$$
$$\mu_{1,2} = 2.94 \text{ or } 3.10 \text{ D}$$

The observed value is about 3.1 D which is in good agreement with that obtained by Tulinskie, Di Giacomo and Smyth[5] in the vapor and dilute benzene solution states. The marked difference between the observed value and $\mu_{1,3}$ indicates that the compound is not *cis*-1,3-dichloro derivative. Then we can conclude that the compound is not the isomer which has the inverted isomer.

Next, we must consider the possibility (1). As for the remaining three isomers of dichlorocyclohexanes which do not possess the inverted isomer, it is easily seen that the compound is not *trans*-1,3- or *cis*-1,4-dichloro derivative. The reasons for this* are as follows:

(1) The value of the moment of *trans*-1,3-derivative which has the structure represented by (1e, 3p) or (1p, 3e) should be equal to that of the (1e, 3e) structure of *cis*-1,3-derivative. Consequently, regarding this isomer we cannot expect such a large moment as observed.

(2) The moment of *cis*-1,4-dichloro derivative should be equal to $\mu_{1,2}$ except for a small difference due to the so-called "induction effect". Therefore, we can say that the compound in question may be this isomer so far as the moment is concerned. However, we have already prepared a dichloro derivative as a by-product of the preparation of *trans*-1,4-dichloro derivative, and studied its vibration spectra and other properties. The spectra are quite different from those shown in Table II. We have concluded from such investigations that the compound mentioned above is not *cis*-1,2-dichloro derivative, but is *cis*-1,4-dichloro derivative. Details of this research will be soon described.

Next, we are in a position to discuss whether or not the compound in question is *cis*-1,2-dichloro derivative, which is the only

5) A. Tulinskie, A. Di Giacomo and C. P. Smyth, *J. Am. Chem. Soc.*, **75**, 3552, (1953).

* It may not be probable that the compound is the 1,4-dichloro derivative. But we cannot immediately see that it is out of the range of possibility.

isomer* that remains to be considered. Upon the basis of vector addition of bond moment it is expected that the moment of *cis*-1,2-dichloro derivative would be equal to $\mu_{1,2}$ calculated above and to that of the (1e, 2e) structure of *trans*-1,2-dichloro derivative, which we have already estimated at 3.1 D.[3] by the investigations of dipole moments. This value and that of $\mu_{1,2}$ agree with the data shown in Table I. The fact that the Raman and infrared spectra agree with each other indicates that the compound is of low symmetry. For comparison the following fact obtained by us should be noted, namely, regarding the infrared and Raman spectra caused mainly by the C—Cl stretching vibration of the (1p, 2p) isomer of *trans*-1,2-dichloro derivative the mutual exclusion rule for a centrosymmetric molecule holds approximately true, because the molecule has locally the center of symmetry as for the two C—Cl bonds—(the total change of polarizability or dipole moment of the molecule with such normal vibrations as mentioned above may be mainly determined by the change of C—Cl bond polarizabilities or bond moments through the vibrations)—although the molecule as a whole is only of C_2 symmetry. Thus we can, beyond any doubt, conclude that the compound in question is *cis*-1,2-isomer, in conformity with the evidence drawn from the "trans-elimination".[1]

* The possibility that the compound is 1,1-dichloro derivative can be left out of consideration, because the moment of the isomer is expected to be equal to $\mu_{1,3}$ calculated above.

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Polarographic Studies on Heterocyclic Compounds. I. Kinetic Current of Isonicotinic Acid

By Yoshinosuke NAGATA and Isamu TACHI

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Introduction

Polarographic studies of pyridine derivatives have been made by several scholars. The reduction mechanism of picolinic, nicotinic and isonicotinic acids, which are monocarboxylic acid of pyridine, have been studied by Tompkins and others[1-6].

The present authors investigated the limiting current of isonicotinic acid.

Experimental Part

A specimen of isonicotinic acid is offered by Prof. Dr. T. Takahashi (the Department of Pharmacy, Faculty of Medicine, Kyoto University).

The concentration of isonicotinic acid in the electrolytic solution, in which 0.1-N potassium chloride is involved as an indifferent electrolyte, is in the range of 10^{-4} to 10^{-3} mol per liter. The *p*H of electrolytic solution is changed by using the various kinds of buffer solutions, which are Mc Ilvain's phosphate buffer, Kolthoff's buffer, and Sörensen's borate buffer. The volume of buffer solution contained in the electrolytic solution corresponds to 80 vol. % of the whole solution.

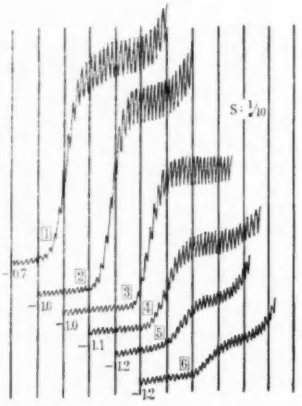


Fig. 1. Polarograms of 5×10^{-4} m./l. isonicotinic acid at various *p*H. (1) 2.2, (2) 7.0, (3) 8.2, (4) 9.0, (5) 10.0, (6) 10.8.

1) M. Shikata and I. Tachi *Chem. News*, **137**, 125 (1928).
2) P. C. Tompkins and C. L. Shmidt *J. Biol. Chem.*, **143**, 643 (1942).
3) P. C. Tompkins and C. L. Schmidt, *Univ. California Pub., Physiol.*, **8**, 229 (1943).
4) P. C. Tompkins and C. L. Schmidt, *Univ. California Pub., Physiol.*, **8**, 237 (1943).
5) P. C. Tompkins and C. L. Schmidt, *Univ. California Pub. Physiol.*, **8**, 247 (1943).
6) E. Knobloch, *Collection*, **12**, 407 (1947).

The Yanagimoto Type Polarograph is used and the normal calomel electrode is connected with the electrolytic solution as a reference electrode. The sensitivity of the galvanometer is 3×10^{-9} Amp./mm./m. The rate of the flow of mercury from the capillary, m, and drop time t, at -1.6 volt vs. N.C.E. are 1.202 mg./sec. and 4.26 sec. at 20°C., respectively.

Experimental Results

(1) Effects of the *p*H on the Half-wave Potential and the Limiting Current.

The polarograms obtained with 5×10^{-4} m./l. isonicotinic acid solution of various *p*H values show a so-called well-defined single reduction wave in the whole *p*H range (Fig. 1). The relationship between *p*H, the half-wave potential, and the limiting current is shown in Table I. As shown by curve (1) in Fig. 2,

TABLE I

RELATIONSHIP BETWEEN THE *p*H, $E_{1/2}$, AND THE LIMITING CURRENT OF THE REDUCTION WAVE OF 5×10^{-4} m./l. ISONICOTINIC ACID

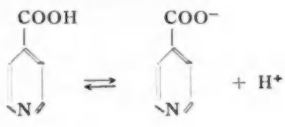
Temperature: 20°C.

*p*H	Buffer	$E_{1/2}$ (vs. N.C.E.)	i(μA)
2.2	Mc Ilvain's buffer	−0.910 v.	2.30
3.0	,,	−0.970	2.30
4.0	,,	−1.030	2.31
5.0	,,	−1.120	2.40
6.0	,,	−1.180	2.40
7.0	,,	−1.280	2.10
6.2	Kolthoff's buffer	−1.190	2.30
7.1	,,	−1.260	2.04
7.5	,,	−1.290	1.83
8.2	,,	−1.330	1.56
8.5	,,	−1.360	1.35
9.0	,,	−1.410	0.93
9.2	Sörensen's buffer	−1.420	0.72
9.5	,,	−1.440	0.48
10.0	,,	−1.460	0.26
10.8	,,	−1.470	0.18
12	,,	−1.470	—
13	in 0.1-N NaOH	—	—

the half-wave potential shifts to more negative value with increasing *p*H value and in the range of *p*H greater than 10 it remains nearly constant.

While the limiting current shows a constant value in the *p*H range from 2 to 6, it decreases gradually with increasing *p*H value in the range of *p*H greater than 7 until it diminishes at *p*H 12.

Isonicotinic acid is a weak mono-basic acid and dissociates as follows:



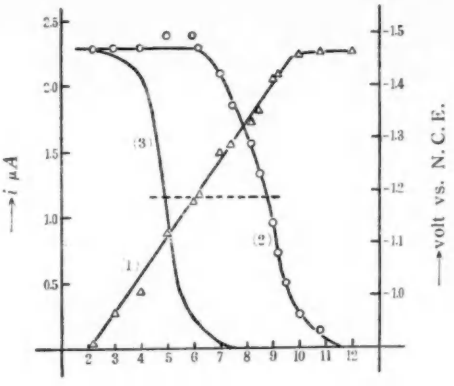


Fig. 2
(1).....$E_{1/2}$-*p*H curve
(2)·····i-*p*H curve
(3).....Dissociation curve

Its dissociation constant, K, is equal to 1.09×10^{-5} [7]. Curve (3) in Fig. 2 represents the dissociation curve of isonicotinic acid and the course of i-*p*H curve (curve (2) in Fig. 2) runs similarly to it. Therefore, provided the reduction wave is due to the reduction of undissociated molecules of isonicotinic acid, curve (2) is to be regarded to show the apparent polarographic dissociation curve.

(2) Effect of the Concentration of Isonicotinic Acid on the Limiting Current.

The relationship between the concentration of isonicotinic acid, the half-wave potential, and the limiting current at *p*H 5.0 at 22°C. is shown in Table II. As shown in column 4 (Table II) and Fig. 3 the limiting current is

TABLE II

RELATIONSHIP BETWEEN THE CONCENTRATION OF ISONICOTINIC ACID AND ITS LIMITING CURRENT AT *p*H 5.0

Temperature: 22°C.

Conc. ($\times10^{-4}$ m./l.)	$E_{1/2}$ (vs. N.C.E.)	i(μA)	i/Conc.
9	−1.050 v.	4.56	0.506
8	−1.040	4.11	0.512
7	−1.030	3.54	0.505
6	−1.040	3.06	0.510
5	−1.030	2.52	0.504
4	−1.030	2.04	0.510
3	−1.020	1.56	0.520
2	−1.020	1.02	0.510
1	−1.030	0.54	0.540

7) H. Scudder, "Conductivities and Ionization Constants of Organic Compounds", 267 (1914).

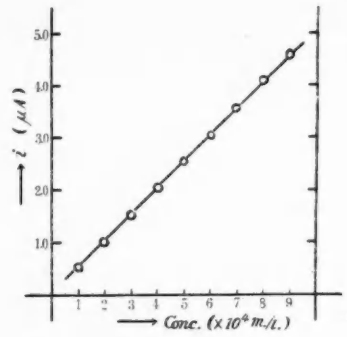


Fig. 3

proportional to the concentration of isonicotinic acid. Hence the quantitative analysis of isonicotinic acid is possible at *p*H 5.0 in the range from 10^{-4} to 10^{-3} mol per liter. The half-wave potential is hardly influenced by the change of concentration (Column 2 in Table II).

(3) Effect of the Height of Mercury Reservoir on the Limiting Current.

Table III represents the relationship between the mercury reservoir height, h, and the limiting current at *p*H 3.0 and 9.5. The fact that the limiting current at *p*H 3.0 is proportional to $\sqrt{h}$ (column 5 in Table III) means that this current is controlled by a diffusion process as shown by the Ilkovič theory[8]; whilst, the limiting current at *p*H 9.5 is independent of h, i.e., it can be concluded that the limiting current at *p*H 9.5 shows the characteristics of kinetic current[9,10].

TABLE III

DEPENDENCE OF THE HEIGHT OF MERCURY RESERVOIR ON THE LIMITING CURRENT OF 5×10^{-4} m./l. ISONICOTINIC ACID

Temperature: 20°C.

		*p*H 9.5	*p*H 3.0	
h	$\sqrt{h}$	i	i	$i/\sqrt{h}$
60 cm.	7.75	0.495 μA	2.400 μA	0.31
55	7.42	0.495	2.280	0.31
50	7.07	0.510	2.265	0.32
45	6.71	0.510	2.175	0.33
40	6.33	0.510	2.070	0.33
35	5.92	0.499	1.950	0.33
30	5.48	0.495	1.830	0.33
25	5.00	0.510	1.665	0.33

TABLE IV

INFLUENCE OF THE TEMPERATURE ON THE LIMITING CURRENT OF 5×10^{-4} m./l. ISONICOTINIC ACID

*p*H 3.0			*p*H 9.5	
t°C.	i	Temp.-Coeff.	t°C.	i	Temp.-Coeff.
22.4	2.40 μA		21.2	0.548 μA	
26.4	2.58		25.2	0.630	3.19%
30.0	2.70		30.5	0.705	2.67%
35.7	2.97	Av.	36.2	0.774	2.27%
41.0	3.24	1.62%	39.4	0.819	1.92%
45.5	3.48		45.0	0.870	1.89%
50.5	3.78		50.5	0.910	1.80%
55.2	4.08		55.5	0.975	1.63%

(4) Effect of the Temperature on the Limiting Current.

The relationship between the temperature and the limiting current at *p*H 3.0 and 9.5 is shown in Table IV. The temperature coefficient of the limiting current which is expressed by $\left(\frac{1}{i}\frac{di}{dT}\right)\times100\%$ is about 1.6% per degree at *p*H 3.0 and it is equal to that of the diffusion current[11]. The coefficient at *p*H 9.5 is greater than at *p*H 3.0 (Column 6), but it decreases gradually with rising temperature. This tendency seems to be opposite to the general one concerning the temperature coefficient. The reason why such a phenomenon presents itself cannot be interpreted and must to be investigated furthermore.

Discussion

As the limiting current at *p*H 3.0 is controlled by diffusion process, the theoretical diffusion current can be calculated from Ilkovič's equation[8]

8) D. Ilkovič *Collection*, **6**, 498 (1934).
9) R. Brdička and K. Wiesner, *Collection*, **12**, 138 (1947).
10) J. Koutecký and R. Brdička, *Collection*, **12**, 337 (1947).
11) D. Ilkovic, *Collection*, **10**, 249 (1938).

$$i_d = 0.605nFCD^{1/2}m^{2/3}t^{1/3} \quad (1)$$

where i_d denotes the diffusion current, n electron number involved in the reduction, F Faraday's constant, C the concentration of the depolarizer, D its diffusion coefficient, m the rate of flow of mercury from the capillary, and t drop time. Employing the following numerical values: $C=5\times10^{-4}$ m./l., $D=8.86\times10^{-6}$ (assuming that the diffusion coefficient of isonicotinic acid is equal to that of benzoate[12]), $m=1.201$ mg./sec., $t=4.26$ sec., and supporting that $n=2$, we obtain the result $i_d=2.514$ μA. Hence the experimental value agrees nearly with the calculated value within experimental error. Therefore, the reduction of isonicotinic acid seems to correspond to the two electron reduction.

Next, as the result of the wave analysis, the reduction process of isonicotinic acid is found to be irreversible; and this conclusion is also confirmed from the standpoint of A. C. polarography, in which A.C. voltage of an arbitrary amplitude is superposed on the D.C. voltage of polarograph, and A.C. component of the pulsating current resulting electrolysis shows a peak at the half-wave potential of polarographic wave in the case of reversible system, while such a peak does not appear in the case of irreversible system[13,14].

The kinetic current due to the recombination of the dissociated anion with proton was for the first time studied theoretically by R. Brdička and K. Wiesner[9]. Later, J. Koutecký and R. Brdička[10] cleared it up mathematically.

In the dissociation equilibrium of isonicotinic acid

$$HA \underset{k_1}{\overset{k_2}{\rightleftarrows}} H^+ + A^- \quad (2)$$

where HA, A^-, and H^+ denote the undissociated molecule, dissociated anion, and hydrogen ion, respectively, dissociation constant, K, is given by

$$K = k_2/k_1 = [H^+][A^-]/[HA] \quad (3)$$

where k_1 and k_2 are the recombination and dissociation velocity constant, respectively. In the reduction of acid, the undissociated molecule is generally reduced at the more positive potential than the dissociated anion. The reduction wave in the case of isonicotinic acid is considered to be due to the reduction of undissociated molecules and the anion reduction wave seems to be overlapped with the wave of indifferent cation or hydrogen ion in the electrolytic solution. The difference between the dissociation curve (curve (3) in Fig. 2) and the apparent polarographic dissociation curve (curve (2) in Fig. 2) is clearly caused by the kinetic current due to the recombination of dissociated anion with hydrogen ion.

According to J. Koutecký and R. Brdička[10], the change of the concentration of both molecule and anion with time is represented by the algebraic sum of diffusion and recombination terms; that is

$$\frac{\partial a}{\partial t} = D\frac{\partial^2 a}{\partial x^2} + \rho(b - \sigma a) \quad (4)$$

$$\frac{\partial b}{\partial t} = D\frac{\partial^2 b}{\partial x^2} - \rho(b - \sigma a) \quad (5)$$

where a and b the concentration of undissociated molecule and dissociated anion, respectively, and

$$\rho = k_1[H^+], \quad \sigma = K/[H^+]$$

The proper initial and boundary conditions are as follows;

$$a = a_e, \quad b = b_e \quad \text{for} \quad t=0, \quad \dot{x}>0$$
$$a = 0, \quad \partial b/\partial x = 0 \quad \text{for} \quad t>0, \quad x=0$$

where a_e and b_e are the equilibrium concentration of a and b, respectively. Eq. 4 can be integrated by means of Laplace transformation, and then the solution is given by

$$\frac{\sqrt{\pi D}}{\alpha}\left(\frac{\partial a}{\partial x}\right)_{x=0} = \frac{1}{(\sigma^2-1)}\left[t^{-1/2}(\sigma e^{-\rho(1+\sigma)t}-1) + 2\frac{\rho^{1/2}\sigma^2}{(\sigma^2-1)^{1/2}}e^{\frac{\rho t}{\sigma-1}}\left\{\int_0^{\sigma\sqrt{\frac{\rho t}{\sigma-1}}} e^{-x^2}dx - \int_0^{\sqrt{\frac{\rho t}{\sigma-1}}} e^{-x^2}dx\right\}\right] \quad (6)$$

where α denotes the analytical concentration of acid. When $\sigma=0$, i.e., dissociation does not take place at all, Eq. 6 is simplyfied to

$$\left(\frac{\partial a}{\partial x}\right)_{x=0} = \frac{a_e}{\sqrt{\pi Dt}} \quad (7)$$

When $\rho \gg 1$ and $\sigma \gg 1$, Eq. 6 is reduced to

$$\left(\frac{\partial a}{\partial x}\right)_{x=0} = \alpha\sqrt{\frac{\rho}{D\sigma}}e^{\frac{\rho t}{\sigma}}\left[1 - \Phi\left(\sqrt{\frac{\rho t}{D}}\right)\right] \quad (8)$$

where

$$\Phi(y) = \frac{2}{\sqrt{\pi}}\int_0^y e^{-y^2}dy$$

and when $\rho \gg 1$, $\sigma \gg 1$, and $\rho/\sigma \gg 1$, Eq. 6 is simplyfied to

$$\left(\frac{\partial a}{\partial x}\right)_{x=0} = \alpha\sqrt{\frac{\rho}{D\sigma}} \quad (9)$$

Therefore, from Eq. 9 and the surface area

12) M. J. Astle and W. V. Mc Connell, *J. Am. Chem. Soc.*, **65**, 35 (1943).
13) B. Breyer, F. Gutmann and S. Hacobian, *Aust. J. Sci. Res.*, **A3**, 558 (1950).
14) I. Tachi, F. Kambara and M. Senda, not published yet.

of mercury drop $q=0.85\,m^{2/3}t^{2/3}$, the average kinetic current, $\bar{i}$, is shown by

$$\bar{i}=\frac{3}{5}\cdot 0.85m^{2/3}t_1^{2/3},nF\alpha\sqrt{\frac{D\rho}{\sigma}} \qquad (10)$$

Since $3/5\times 0.85m^{2/3}t_1^{2/3}$, in Eq. 10 represents the average surface area of the dropping mercury electrode and the product mt_1 is independent of the height of mercury reservoir, when the same capillary is used under the same conditions, the kinetic current shows a constant value independently of the change of the mercury reservoir height. From Eq. 8, the average kinetic current is given by

$$\bar{i}=\frac{0.85m^{2/3}nF\alpha}{t_1}\int_0^{t_1}\sqrt{\frac{D\rho}{\sigma}}e^{\frac{\rho t}{\sigma}}\left[1-\Phi\left(\sqrt{\frac{\rho t}{\sigma}}\right)\right]t^{2/3}dt \qquad (11)$$

when $k_1\to\infty$, the limiting current has no character of the kinetic current, but corresponds to the diffusion current. Denoting it by $\bar{i}_\infty$, from Eqs. 7 and 11 it is seen that

$$\frac{\bar{i}}{\bar{i}_\infty}=\frac{6}{7\sqrt{\pi}y^{7/6}}\int_0^{y_1}F(y)dy=f(y_1) \qquad (12)$$

where

$$F(y)=y^{2/3}e^y[1-\Phi(\sqrt{y})]$$
$$y=\rho t/\sigma,\quad y_1=\rho t_1/\sigma \qquad (13)$$

When the kinetic current corresponds to a half of the diffusion current, it can be shown that

$$f(y_1)=0.5$$

and then

$$y_1=0.420(*)$$

When $K\gg[H^+]$, it follows from Eq. 13 that

$$y_1=\frac{k_1[H^+]}{K}t_1$$

$$\log k_1=2\left(p\mathrm{H}+\frac{1}{2}\log y_1\right)-(pK+\log t_1) \qquad (14)$$

The pH value where $f(y_1)=0.5$ in curve (2) (Fig. 2) is 8.8, and $t=4.26$ sec. and

$$K=1.09\times 10^{-5}.$$

Substituting these values into Eq. 14, the recombination velocity constant is equal to

$$k_1=1.22\times 10^{12}\ \text{sec.}^{-1}\ (\text{m./l.})^{-1}$$

Further, it is found that the dissociation velocity constant is

$$k_2=k_1K=1.33\times 10^7\ \text{sec.}^{-1}$$

According to J. Koutecký and R. Brdička[10], the thickness of the reaction layer where recombination takes place, μ, is given by

$$\mu=\sqrt{\frac{D}{k_1K}} \qquad (15)$$

Inserting the above mentioned values viz., $D=8.86\times 10^{-6}$, $k_1=1.22\times 10^{12}$ and $K=1.09\times 10^{-5}$, into this equation, it is found that

$$\mu=8.16\times 10^{-7}\ \text{cm.}$$

This value seems to be right in comparison with the other values obtained experimentally concerning some organic acids[17,18].

Summary

(1) Isonicotinic acid shows a single polarographic reduction wave. It is found from the standpoint of the wave analysis as well as of A.C. polarography that the electrode process is irreversible.

(2) The wave height at pH 5.0 is proportional to the concentration of isonicotinic acid in the concentration range from 10^{-4} to 10^{-3} mol per liter.

(3) Since the single reduction wave is considered to be due to the reduction of undissociated molecules, the i-pH curve shows an apparent polarographic dissociation curve.

(4) The kinetic current due to the recombination of dissociated anions with proton is investigated, and the recombination velocity constant and the thickness of reaction layer are determined according to J. Koutecký and R. Brdička's equations.

The authors would like to thank Prof. Dr. T. Takahashi for gifts of isonicotinic acid used in this work. We are also indebted to M. Suzuki, F. Kambara, M. Senda, and S. Koide for their suggestions.

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*) According to J. Koutecký and R. Brdička[10], y_1 is equal to 0.400; but J. Koryta[15] points out the error as to this value. M. Senda[16] newly obtains the plot of y_1 vs. f(y_1) from the numerical calculation, and the above value is obtained from this plot.

15) J. Koryta, *Proc. I.Inter. Polaro. Congr. in Prague,* **1** 798 (1951).
16) M. Senda; not published yet.
17) R. Brdička, *Proc. I. Inter. Polaro. Congr. in Prague,* **3** 286 (1952).
18) I. M. Kolthoff and A. Liberti, *J. Am. Chem. Soc.*, **70**, 1885 (1948).

The Electrostatic Effect of the Solvents on the Frequency Shifts and the Intensity Changes of the Infrared Absorption Spectra

By Eizi HIROTA

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Recently a theory was presented by the author[1] on the frequency shifts of the infrared absorption bands in the liquid state in terms of the intermolecular potential by Onsager's theory of dielectrics. The treatment given there, however, was essentially classical, so that it may be desirable to reconsider it in quantum mechanics.

For simplicity let us take a heteronuclear diatomic molecule with one degree of freedom of vibration which may be expressed approximately by a harmonic oscillation. As was stated in the preceding paper[1] the molecules in the liquid are stabilized by the interaction with the surrounding molecules and the energy to be added is given by[2]

$$\Delta H = -\frac{\epsilon-1}{2\epsilon+1}\frac{C}{a^3}[\mu+e'Q]^2 \quad \text{.....(1)}$$

It is easily shown that the first order perturbation energy due to the operator, Eq. (1), gives rise to

$$\int \psi_0 \Delta H \psi_0 d\tau = -\frac{\epsilon-1}{2\epsilon+1}\frac{C}{a^3}\left[\mu^2+\frac{e^2h}{8\pi^2 m\nu}\right] \quad \text{.....(1a)}$$

for the ground state and

$$\int \psi_1 \Delta H \psi_1 d\tau = -\frac{\epsilon-1}{2\epsilon+1}\frac{C}{a^3}\left[\mu^2+\frac{3e^2h}{8\pi^2 m\nu}\right] \quad \text{.....(1b)}$$

for the first excited state, respectively. There ψ_0 and ψ_1 denote the wave functions of the ground and the first excited states of the harmonic oscillator. Then the energy absorbed in the transition, 0→1, is given by

$$h\nu' = h\nu - \left[\int \psi_0 \Delta H \psi_0 d\tau - \int \psi_1 \Delta H \psi_1 d\tau\right] = h\nu\left[1-\frac{\epsilon-1}{2\epsilon+1}\frac{C}{a^3}\frac{e^2}{4\pi^2 m\nu^2}\right] \quad \text{...(2)}$$

Thus the decrease in frequency observed at the transfer from the gas to the liquid state becomes

$$\frac{\Delta\nu}{\nu} = \frac{\nu-\nu'}{\nu} = \frac{\epsilon-1}{2\epsilon+1}\frac{C}{a^3}\frac{e^2}{4\pi^2 m\nu^2}, \quad (2')$$

where

$$C = \frac{(n^2+2)(2\epsilon+1)}{3(2\epsilon+n^2)} \quad \text{.....(3)}$$

This is exactly the same expression as that obtained by the classical consideration in the preceding paper.

The intensity of this absorption band depends upon the squared value of the (0, 1) element of the electric moment, while the latter in the liquid state is increased by the factor C referred to that in *vacuo*;

$$m = C[\mu+e'Q] \quad \text{.....(4)}$$

Therefore the absorption intensity in the liquid state, I_l, is obtained from that in the gaseous state, I_g, by the relation

$$I_l = C^2 I_g \quad \text{.....(5)}$$

To test the above conclusions the recent measurement by Barrow[3] is quoted here. The absorption of the stretching frequency of C=O group of various compounds were measured in the solutions of chloroform and carbon tetrachloride as well as in the vapor. Here are selected the following four examples: acetone, methyl and ethyl acetate, and methyl carbonate. In order to compute the frequency shifts by Eq. (2'), it is necessary to know the values of a's, the effective radii of the molecules. They are usually estimated from the density of the liquid, but in this paper they are treated as adjustable parameters so as to give the best fit with the observed ones in $CHCl_3$ and CCl_4 solutions. In Table I the calculated $\Delta\tilde{\nu}$'s are given to be compared with the observed values. In this table are listed the values a's assumed, together with the frequencies in *vacuo* $\tilde{\nu}$'s (in the unit of cm^{-1}), and the intensities in *vacuo* A's quoted from the paper by Barrow. It is noted that, since $A = N\pi\left(\frac{\partial\mu}{\partial Q}\right)^2/3c^2 \ 1000$ given in the unit of $mole^{-1}$ litre cm^{-1} is used, Eq. (2') becomes

1) E. Hirota, This Bulletin, **26**, 397 (1953).

2) Eq. (4*b*) in reference 1, in which the energy necessary to polarize the molecule, $\alpha R^2 2$, was omitted. (Cf. C. J. F. Böttcher, "Theory of Electric Polarization", Elsevier Publishing Co., Amsterdam, 1952, pp. 138—139.) This correction being included, the factor C^2 in Eq. (4*b*) in reference 1 should be replaced by C, but it does not essentially alter the conclusion obtained there. C^2 in Eqs. (6*b*), (7*b*), (8*b*) and (8*b'*), should also be corrected to C. About notations used in this paper one should consult with referrence 1.

3) G. M. Barrow, *J. Chem. Phys.*, **21**, 2008 (1953).

TABLE I
FREQUENCY SHIFT

Compound	a (A)	$\tilde{\nu}$(cm^{-1})	A*)	$CHCl_3$ soln. $(\epsilon-1)C/(2\epsilon+1)=0.444$ calc.	obs.	CCl_4 soln. $=0.253$ calc.	obs.
Acetone	1.48	1747	$1.2\,10^4$	(38.0)	38	21.3	—
Methyl acetate	1.68	1776	1.9	40.3	38	23.0	25
Ethyl acetate	1.73	1772	2.1	41.7	38	23.2	27
Methyl carbonate	2.20	1781	2.9	27.2	25	15.5	18

*) $A=N\pi\left(\frac{\partial\mu}{\partial Q}\right)^2/3c^2\ 1000$, given in the unit of mole^{-1} litre cm^{-1}.

$$\Delta\tilde{\nu}=\frac{\epsilon-1}{2\epsilon+1}C\frac{3}{4\pi a^3}\frac{10^3}{N\pi^2}\frac{A}{\tilde{\nu}} \qquad ...(2'')$$

The values of dielectric constants are taken to be 5.0 for $CHCl_3$ and 2.23 for CCl_4, while $n^2=2$ is assumed through out. It follows that $(\epsilon-1)C/(2\epsilon+1)=0.444$ for $CHCl_3$ and 0.253 for CCl_4 solutions. For the last three compounds in Table I. the calculated values of $\Delta\tilde{\nu}$'s for $CHCl_3$ solutions are larger than the experimental values, whereas the contrary is the case for CCl_4 solutions.

The values assumed above for a's are found to be far smaller than the molecular radius $a_0=(3M/4\pi Nd)^{1/3}$, which is the radius of the spheres just filling the entire liquid. The bond moments of C=O and C−O are considered to be greater than those of C−C and C−H, so that the interactions of the bonds C=O and C−O with other molecules may play the main role in this case. The radius of a sphere encircling just this group of oxygen and carbon atoms is taken to be the rough estimate for a. The atomic radii and the bond angles of acetate and carbonate groups are taken from Pauling's book[4], where the two end oxygen atoms in acetate groups are assumed to have radii $0.605=(0.66+0.55)/2$ and the three in carbonate group to have radii $0.623=(2\times0.66+0.55)/3$. The values obtained thus are 1.28A for acetone, 1.75A for two acetates and 2.25A for carbonate. It may be interesting to note that a's used for the calculation of $\Delta\tilde{\nu}$ 1.48A, 1,68A, 1.73A and 2.20A run roughly parallel to these rough estimates.

Another comparison is carried out for the intensity change. As evident from Eq. (5). the intensity ratio I_l/I_g is given by C^2, which depends on ϵ and n, but is free from the value of a. Inserting the values of dielectric constants for $CHCl_3$ and CCl_4 as quoted above and using the intensities in *vacuo* I_g measured by Barrow, one can obtain I_l, the intensities in solutions, which are listed in Table II. It will be seen that the agreement with the observed I_l is very satisfactory.

TABLE II
INTENSITY CHANGE (1)

Compound	A_g*)	A_l*) $C^2=1.49$ $CHCl_3$ soln. calc.	obs.	$C^2=1.265$ CCl_4 soln. calc.	obs.
Acetone	$1.2\,10^4$	$1.79\,10^4$	$1.9\,10^4$	$1.52\,10^4$	...
Methyl acetate	1.9	2.83	2.8	2.40	$2.2\,10^4$
Ethyl acetate	2.1	3.13	3.2	2.65	2.6
Methyl carbonate	2.9	4.33	4.2	2.67	3.8

*) See the footnote of Table I.

Further it is also possible to compare the intensities in different solutions; the ratio of the intensities in two solutions is given by

$$I_{li}/I_{lj}=C_i^2/C_j^2 \qquad .....(6)$$

where C_i and C_j denote respectively the constants for both solutions. Thus the intensity ratio is a definite constant, determined by the solvents, and common to any absorption bands of any melecules. In Table III. a comparison is made of the intensity ratios $\rho=A\ (CHCl_3)/A(CCl_4)$ of C=O bands of various compounds in chloroform and carbon tetrachloride. It will be clearly seen that the intensity ratios are obtained in the deviations less than 10% from the calculated ratio $\rho_{calc.}=C^2_{(CHCl_3)}/C^2_{(CCl_4)}=1.18$, except in the cases of propionaldehyde (14.7%) and ethyl benzoate (10.1%). According to Barrow, the accuracy of the intensity measurement is about 2% in solutions and about 5% in the gaseous phase. The discrepancies between the

4) L. Pauling, "The Nature of the Chemical Bond" Cornell Univ. Press, 1940, pp. 164, 203, 208 and 209.

TABLE III
INTENSITY CHANGE (2)

Compound	$A_{obs.}$ *) $CHCl_3$ soln.	CCl_4 soln.	$\rho_{obs.}=A(CHCl_3)/A(CCl_4)$	$\frac{\rho_{obs.}-\rho_{calc.}}{\rho_{calc.}}$ Deviation in ρ (%)
Diethyl ketone	$1.9\ 10^4$	$1.55\ 10^4$	1.2_2	3.7
Propionaldehyde	2.1	1.55	1.3_5	14.7
Benzaldehyde	2.6	2.1	1.2_4	5.1
Benzophenone	2.4	2.2	1.0_9	−7.4
Acetophenone	2.7	2.1	1.2_8	9.0
Ethyl propionate	3.2	2.8	1.1_4	−3.1
Methyl crotonate	3.4	3.1	1.1_0	−6.9
Ethyl benzoate	3.6	3.4	1.0_5	−10.1
Ethyl carbonate	4.7	4.1	1.1_4	−2.9
Acetyl chloride	3.1	2.5	1.2_3	5.3
Phenyl acetate	3.1	2.9	1.0_7	−9.2
Acetanilide	3.6	3.1	1.1_6	−1.4

$\rho_{calc.}=C^2(CHCl_3)/C^2(CCl_4)=1.49/1.265=1.18$

*) See the footnote of Table I.

calculated and the observed values are of this order of magnitude.

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A Study of the Effect of Fuchsin Red on the Metabolism of Yeasts

By Krishna BAHADUR

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Introduction

In recent years a good deal of work has been done upon the influence of substances which, although not essential as nutrients, still affect the physiological properties of yeast cells[1]. In this paper I have studied the growth and metabolic activity of yeasts in cultures which have the property of absorbing visible light. I selected such a water-soluble dye, which in small doses, does not harm the yeast to a great extent but gives the culture a distinct color. Fuchsin red was chosen as one of such dyes for the study.

These cultures have deep red color. They naturally absorb all the radiations of the visible light except the red light. This absorption of light is followed by simultaneous change in the cells of the cultures. *Chaetomium*[2] and *Serratia* have been found to be mutated by high doses of wave length above 350mμ. The latter bacterium indicates 6 per cent color sector changes by 10^7 erg/cm^2. without noticeable killing. When cells are vitally stained by photodynamic dyes, e. g., erythrosine, the sensitivity to visible light becomes high 3—6).

The influence of dyeing time, concentration, *p*H, and temperature on photodynamic effects can be interpreted in terms of permeability and absorption of the dye. In *Penicillium*[5] and *E. coli* 15 h [7,8] photodynamic and ultraviolet induced mutation processes are similar regarding close curve and correlation to killing. Phage resistance[9] and Sd 4 mutations[10] are also induced by photodynamic action.

1) W. A. Krehl, and S. J. Llao, *Ann. Rev. Microbiol.*, **5**, 121 38 (1951).
2) A. L. Mc Aulay, J. M. Ford, and D. L. Dobie, *Heredity*, **3**, 109 (1949).
3) R. W. Kaplan, *Naturwissenschaften*. **35**, 127 (1948).
4) R. W. Kaplan, *Nature*, **163**, 573 (1949).
5) R. W. Kaplan, *Arch. Microbiol.*, **15**, 152 (1949).
6) R. W. Kaplan, *Planta*, **38**, (1949).
7) R. W. Kaplan, *Naturwissenschaften*, **31**, 547 (1950).
8) R. W. Kaplan, *Microbiol. Genet. Bull.*, **4**, 12, (1951).
9) R. W. Kaplan, *Naturwissenschaften*, **37**, 308, (1950).
10) M. Demerec, G. Bertani, and J. Flint, *Am. Naturalist*, **85**, 119 (1951).

When fuchsin red is present in yeast cultures it stains the cells and effects a change in the physiological properties of yeasts. To study this change varying amounts of fuchsin red were taken in yeast cultures containing sucrose as source of carbon and ammonium sulfate as source of nitrogen. The metabolic substances formed during the yeast growth were estimated to investigate the effect of the dye in the cultures. These studies were done with *Saccharomyces carlsbergensis* and Dhar Yeast[11]

Experimental

Five cultures, each containing 0.2 g. of calcium carbonate, 0.2 g. of magnesium carbonate, 0.2 g. of sodium chloride, 0.2 g. of potassium sulfate, 0.25 g. of disodium hydrogen phosphate and 2.5 g. of ammonium sulfate were prepared. Above substances were digested in dilute hydrochloric acid. The amount of fuchsin red, as mentioned against each culture in the table of *Saccharomyces carlsbergensis*, was added in them. This was done by making a standard solution of fuchsin red and then taking out a certain volume of this solution containing the requisite amount of fuchsin and introducing it in the culture solution. Total volume of each culture was made to 400 cc. with distilled water and *p*H adjusted to 4.5. These cultures were kept in 750 cc. flatbottom pyrex flasks and 20 g. of sucrose were added in each of them. The flasks were cotton plugged and sterilised by heating in an autoclave for 20 minutes at 10 lbs. pressure. Cultures were cooled and seeded with activated sample of *Saccharomyces carlsbergensis*.

Four cultures, each containing the requisite minerals in the above mentioned amounts, were prepared and the amount of fuchsin red mentioned against each culture in the table of Dhar Yeast was added in each of them by the procedure mentioned above. These cultures were kept in 500 cc. flatbottom pyrex flasks and the total volume of each culture was made 200 cc. To each of these cultures 20 g. of sucrose were added as the source of carbon. These cultures were sterilised as mentioned above and after cooling they were seeded with a trace of an activated sample of Dhar Yeast.

Observation

Following tables show the result of the effect of fuchsin red on the growth of yeast, alcohol formation, acid production and sugar consumption in 5 per cent (w/v) sucrose solution using *Saccharomyces carlsbergensis* variety of yeast under non-aerated conditions. The *p*H of the cultures was 4.5 in the beginning and the temperature variations during the period of fermentation was between 29.4 to 32.4°C.

These cultures were analysed after 18 days. The figures mentioned in the following tables are for 100 cc. of the cultures.

TABLE I

Serial Number	Fuchsin dye added in the culture (mg.)	Reducing sugar left in the culture after fermentation (g.)	Total sugar left in the culture (g.)	Sugar consumed during fermentation (g.)	Percentage of sugar consumed calc. on the basis of sugar originally present.
1	0.25	2.13	2.25	2.75	55.00
2	0.50	1.85	1.99	3.01	60.20
3	1.00	1.58	1.66	3.34	66.80
4	2.00	1.31	1.33	3.67	73.40
5	4.00	0.00	0.00	5.00	100.00

TABLE II

Serial no. according to Table I.	Total acid formed during fermentation (g. equiv.)	Percentage of acid formation calc. on the basis of sugar consumed	Ethyl alcohol formed during fermentation (g.)	Percentage of alcohol formation calc. on the basis of sugar consumed	Dry yeast grown in the culture (g.)	Percentage of yeast yield calc. on the basis of sugar consumed
1	0.0238	0.86	0.64	23.27	0.4284	15.57
2	0.0524	1.74	0.71	23.65	0.4148	13.76
3	0.0619	1.82	0.96	28.74	0.3938	11.79
4	0.0762	2.07	1.39	37.87	0.3888	10.59
5	0.2142	4.28	2.01	40.20	0.3288	6.57

11) N. R. Dhar, and Krishna Bahadur., *The Proceedings of the National Acad. of Sci.*, Vol. 19, Part II, 55–9 (1950).

Following tables indicate the results of the effect of fuchsin red on Dhar Yeast cultures containing 10 percent (w/v) of sucrose as source of carbon and ammonium sulphate as nitrogen source, under non-aerated conditions. These cultures were analysed after 10 days and the temperature variation during the period of fermentation was between 26.4° to 29.8°C. The *p*H of the cultures in the beginning was 4.5. The results mentioned below are for 100 cc. of the cultures.

TABLE III

Serial No.	Fuchsin added in the culture (mg.)	Total sugar left in the culture after fermentation (g.)	Reducing sugar left in the culture (g.)	Nonreducing sugar left in the culture (g.)	Sugar consumed during fermentation (g.)	Percentage of sugar consumed calc. on the basis of sugar originally present
1	0.125	8.36	6.86	1.50	1.64	16.4
2	0.250	7.76	6.86	0.90	2.24	22.4
3	0.500	6.86	6.85	0.01	3.14	31.4
4	1.000	7.66	7.18	0.48	2.82	28.2

TABLE IV

Serial No. according to Table III	Total acid formed during fermentation (g. equiv.)	Percentage of acid formation calc. on the basis of sugar consumed during fermentation	Ethyl alcohol formed (g.)	Percentage of alcohol formation calc. on the basis of sugar consumed	Dry yeast grown during fermentation (g.)	Percentage of yeast yield calc. on the basis of sugar consumed
1	0.4324	26.21	0.69	44.07	0.7356	44.85
2	0.4232	18.75	0.58	25.89	0.7440	33.21
3	0.4232	13.37	0.53	16.87	0.7694	24.50
4	0.4232	15.24	0.44	15.67	0.7862	24.36

Inference

Dhar Yeast shows a marked decrease in alcohol forming property up to the concentration of 5×10^{-4} per cent of fuchsin red in the culture after which this decrease becomes constant. *Saccharomyces carlsbergensis* indicates a rapid increase in alcohol forming property up to the concentration of 2×10^{-3} per cent of fuchsin in the culture after which this rise becomes gradual and slow.

The acid producing property of Dhar Yeast rapidly decreases up to the concentration of 5×10^{-4} per cent of fuchsin in the culture after which there is an increase in the acid production; where as *Saccharomyces carlsbergensis* shows a gradual increase in the acid forming property from the very beginning. This increase is gradual up to the concentration of 2×10^{-3} per cent of fuchsin in the culture after which the production of acid increases very rapidly.

The sugar consuming property of Dhar Yeast increases gradually up to the concentration of 5×10^{-4} per cent of fuchsin in the culture after which this shows an upward trend. In the case of *Saccharomyces carlsbergensis* the sugar consuming property gradually decreases with the increase of fuchsin concentration in the culture.

The yield of dry yeast decreases in both cases. Dhar Yeast indicates a more sudden fall but *Sacchoromyces carlsbergensis* shows a very gradual decrease in the yeast yield.

Thus we see that there is a correlation between the radiation induced and spontaneous change in the physiological properties of the microorganism. The different effects of the same influencing factor on different organisms is in accordance with the observation made in the case of *E. coli* whose one strain, particularly streptomycin dependent strain frequently changes spontaneously and after ultra violet radiation, whereas another strain was stable in both respects[12].

Summary

When fuchsin dye is added in the yeast cultures it causes a change in the physiological properties of the yeasts which is characterstic of the variety of yeast used. For

12) M. Demerec, B. Wallace, E. M. Witkin, and G. Bertani, *Carnegie Inst. Wash. Yearbook*, **48**, 154 (1949).

many properties this change becomes more gradual as the concentration of dye increases in the culture. Although there is a vast difference in the nature of change of alcohol forming property of the yeasts studied above, changes of the other properties show a similar trend in both the yeasts under examination, when higher concentrations of fuchsin red is present in the cultures.

The presence of fuchsin in the culture is poisonous to the yeasts. This is indicated by the fact that it hinders their growth. This hindrance is directly proportional to the amount of dye in the culture.

I am much thankful to Prof. N. R. Dhar for his valuable advice in this work.

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Stoichiometric Investigations of the Liquid State. I. The Relation between the Sound Velocity and the Chemical Structure. (1). The Physical Meaning of the Molar Sound Velocity of the Unassociated Liquid

By Kiyoshi TYUZYO[1]

(Received April 17, 1954)

Introduction

Thermodynamic quantities such as adiabatic compressibility ($\beta_{ad}=1/\rho u^2$, where ρ is density, u is ultrasonic velocity in the liquid) and ratio of specific heat ($\gamma=\beta_{iso}/\beta_{ad}$ where β_{iso} is isothermal compressibility of liquid) can easily be evaluated from the ultrasonic velocity in the liquid. Therefore, the measurement of the ultrasonic velocity in the liquid is important to clarify the intermolecular force in the liquid[2].

On the other hand, in 1940, Rao found that K, expressed in the following equation, is a characteristic constant of each liquid and is independent of temperature[3].

$$K=u^{1/3}V \qquad (1)$$

where V is molar volume of the liquid.

Moreover, in 1941, Rao found that K in Eq. 1 can be given as the sum of increments of the constitutive elements[4]. That is, K, as well as parachor and molar refraction, is independent of temperature and has the additive property.

K is now designated as molar or molecular sound velocity. It is now possible from this investigation to treat quantitatively the relation between sound velocity and the chemical structure of liquid. Thereafter, investigations of this field were remarkably developed and were dealt with very strictly by Lagemann[5], Weissler[6], Schaafs[7], etc. But the theoretical investigation of molar sound velocity has scarcely ever been performed[7a].

The present author can satisfactorily interpret the physical meaning of molar sound velocity (K), its temperature-invariance, and its additive property on the basis of the simple theory of liquid state, which will be reported in the following series of papers.

The Physical Meaning of Molar Sound Velocity, K

According to Kincaid and Eyring[8], the sound velocity in a liquid (u) is expressed by the following equation,

$$u=u_{gas}\left(\frac{v}{v_f}\right)^{1/3}=\left(\frac{v}{v_f}\right)^{1/3}\left(\frac{RT\gamma}{M}\right)^{1/2} \qquad (2)$$

where u_{gas} is sound velocity in a gas, v is molecular volume, and v_f is free volume of a liquid.

v_f is approximately given by the following equation[8],

$$v_f=\left(\frac{CRT\,V^{1/3}}{N^{1/3}E_{vap}}\right)^3, \qquad (3)$$

where E_{vap} is the energy of vaporization and

1) Present Address: Research Laboratory, Dai-Nippon Celluloid Co. Ltd., Sakai City, Osaka.
2) L. Bergmann, "Der Ultraschall" (1949).
3) M. R. Rao, *Indian J. Phys.*, **14**, 109 (1940).
4) M. R. Rao, *J. Chem. Phys.*, **9**, 682 (1941).
5) R. T. Lagemann and W. S. Dunbar, *J. Phys. Chem.*, **49**, 428 (1945), etc.
6) A. Weissler, J. W. Fitzgerald and I. Resnick, *J. Appl. Phys.*, **18**, 434 (1974), etc.
7) W. Schaafs, *Z. phys. Chem.*, **A 194**, 28 (1944), etc.
7a) Recently, some theoretical explanation of the Rao's relation has been given by O. Nomoto and T. Kishimoto on the basis of the Lennard-Jones, Devonshires' model: *J. Phys. Soc. Japan*, **9**, 59, 66, 73 (1954).
8) J. F. Kincaid and H. Eyring, *J. Chem. Phys.*, **6**, 620 (1938).

C is the packing number of liquid. (This is equal to 2 for cubic packing.)

Then, u is expressed by Eq. 4, making use of Eq. 2 and 3.

$$u = \frac{E_{vap}\,\gamma^{1/2}}{C(MRT)^{1/2}} \tag{4}$$

Therefore,

$$u^{1/3}\,V = \left[\frac{E_{vap}\,\gamma^{1/2}}{C(MRT)^{1/2}}\right]^{1/3} V \tag{5}$$

In comparison of Eq. 5 and Rao's Eq. 1, the molar sound velocity is expressed as follows,

$$K = \left[\frac{E_{vap}\,\gamma^{1/2}}{C(MRT)^{1/2}}\right]^{1/3} V \tag{6}$$

Namely, Rao's characteristic constant, K, as is shown in Eq. 6, contains several factors such as geometrical configuration of the liquid state (C, V), thermodynamic property of the gaseous state (γ), and property of equilibrium state between liquid and gas (E_{vap}). Therefore, it is rather questionable to conclude simply the additivity of K as was done by Rao[3] and Lagemann[9]. The author will discuss this conclusion in the second paper of this series.

Temperature-invariance of K

In Eq. 6, $E_{vap}{}^{1/6}$, $\gamma^{1/3}$, $C^{1/3}$ and V are dependent on temperature. But, among them, temperature-variance of $\gamma^{1/3}$ is negligible and $C^{1/3}$ can be regarded as temperature-invariable in the range of ordinary temperature (ca. 0° to 100°C). C must be equal to 2 for the cubic packing. For normal unassociated liquids, this value was computed from the vapor pressure data by Eyring and others and is approximately equal to 2. The present author has examined the temperature-variance of C by calculating it from Eq. 5, using the observed value of u, and found that its temperature-variance does not exceed several percent in the range of 50°C for normal liquids. Examples are shown in Table I. Table II shows the comparison of C values obtained from sound velocity and from vapor pressure. The coincidence of the values from two different methods is satisfactory and both methods give $C=2$ on the average, but in general, the value from sound velocity is somewhat smaller than that from vapor pressure.

As no satisfactory theoretical equation for the temperature-variance of E_{vap} and V can be found, so their values at each temperature were obtained from International Critical Table and other tables.

9) R. T. Lagemann, J. S. Evans, and D. R. McMillan, Jr, *J. Amer. Chem. Soc.*, **70**, 2696 (1948).

TABLE I
THE TEMPERATURE-INVARIANCE OF PACKING NUMBER, C

Temperature (°C)	C
Benzene	
10	1.80
20	1.80
30	1.80
40	1.80
50	1.81
60	1.81
Chloroform	
0	1.82
10	1.82
20	1.81
30	1.81
40	1.81
50	1.81
60	1.81

TABLE II
PACKING NUMBER, C, OF NORMAL LIQUIDS

Substance	C from Sound Velocity	C from Vapor Pressure[10]
n-Heptane	——	1.60
n-Octane	1.61	——
Ethyl ether	1.89	1.92
Benzene	1.80	2.12
Acetone	2.09	1.76
Carbon disulfide	1.76	2.05
Ethyl bromide	1.89	——
Ethylene chloride	1.81	——
Chloroform	1.81	2.44
Carbon tetrachloride	1.77	2.08

γ is the value from the Taschenbuch[11], but in the case of the compound of which γ is unknown, its value is approximately obtained from the following method. As the first approximation, γ-values are determined by the number of atoms constructing the molecule, then the relation between known values of γ and numbers of atoms constructing the molecule is drawn in Fig. 1. As is

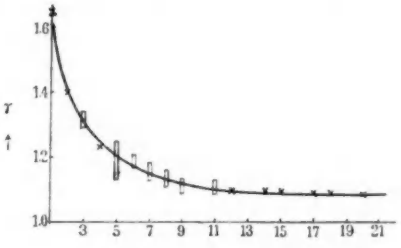

Fig. 1. The relation between the ratio of specific heats (γ) and the number of atoms constituting the molecule in the gaseous state.

10) H. Eyring and J. Hirschfelder, *J. Phys. Chem.*, **41**, 249 (1937).

11) J. D'Ans and E. Lax, "Taschenbuch für Chemiker und Physiker" (1949).

shown in the figure, γ varies in the considerably broad range in the molecules which are made of small numbers of atoms, but the values become fixed dependent only upon the numbers of atoms constructing the molecule when the molecules are made of many numbers of atoms. If the experimental values are connected with a smooth curve as in the figure, γ can be determined from this curve when the number of atoms constructing the molecule is known. (As γ appears in Eq. 6 with 1/6-power, this degree of approximation is quite satisfactory.)

With these treatments, E_{vap}, γ, C and V-values are known, and so K-values can be calculated by Eq. 6 at several temperatures.

Results are shown in Table III for several normal liquids. As is clear from the table, K is almost independent of temperature and this result is consistent with that of Rao and others.

TABLE III
TEMPERATURE-INVARIANCE OF THE CAICULATED K-VALUES FOR NORMAL LIQUIDS

Temperature (°C)	E_{vap} (cal/mol)	ρ(g./cc.)	K_{calc} (c.g.s.)
	n-Octane, M=114.2, γ=1.05		
0	7660	0.719	7410
20	7620	0.702	7470
40	7580	0.686	7540
	Benzene, M=78.2, γ=1.10		
10	7640	0.890	4370
20	7500	0.879	4360
30	7360	0.868	4360
40	7220	0.858	4370
50	7070	0.847	4370
60	6920	0.836	4370
	Ethyl bromide, M=109.0, γ=1.19		
0	6240	1.501	3260
10	6150	1.480	3270
20	6070	1.461	3250
30	6010	1.440	3290
	Ethylene chloride, M=99.0, γ=1.37		
0	7870	1.281	3780
10	7780	1.267	3780
20	7670	1.251	3780
30	7550	1.238	3790
40	7430	1.223	3790
50	7300	1.209	3800
	Chloroform, M=119.4, γ=1.15		
0	7170	1.525	3620
10	7040	1.506	3620
20	6900	1.487	3610
30	6780	1.467	3620
40	6640	1.449	3620
50	6500	1.429	3620
60	6370	1.409	3630
	Carbon tetrachloride, M=153.9, γ=1.13		
0	7740	1.633	4270
10	7460	1.613	4240
20	7210	1.594	4220
30	7080	1.575	4210
40	7000	1.556	4230
50	6930	1.536	4240
	Acetone, M=58.05, γ=1.1		
0	7260	0.813	3690
10	7170	0.801	3710
20	7050	0.791	3710
30	6920	0.779	3720
40	6790	0.767	3740
50	6640	0.755	3750
	Ether, M=74.1, γ=1.08		
0	6290	0.736	4750
10	6090	0.725	4750
20	5880	0.714	4750
30	5700	0.702	4750
	Methyl acetate, M=74.1, γ=1.14		
10	7570	0.946	3950
20	7300	0.932	3930
30	7070	0.918	3930

Comparison of the Calculated and Observed K-values

It was found in the previous paragraph that K calculated from Eq. 6 is independent of temperature and is the characteristic constant of the substance. Next, the K-values calculated from Eq. 6 and observed (Eq. 1) are compared. Results are shown in Table IV[12]. It is clear from the table,

$$K_{calc}/K_{obs}=1$$

(Accuracy is about ±5%.)

TABLE IV
THE CALCULATED AND OBSERVED MOLAR SOUND VELOCITIES, K

Substance	K_{calc}	K_{obs}	K_{calc}/K_{obs}
n-Hexane	6070	6260	0.969
n-Octane	7470	7990	0.936
Benzene	4360	4530	0.962
Carbon disulfide	2810	2940	0.957
Ethyl bromide	3250	3340	0.973
Ethylene chloride	3780	3910	0.967
Chloroform	3610	3730	0.968
Carbon tetrachloride	4220	4390	0.962
Ether	4750	4830	0.983
Acetone	3710	3610	1.027
Methyl acetate	3930	3890	1.010
Ethyl acetate	4770	4810	0.992
Ethyl formate	3260	3730	0.874
Ethyl iodide	3480	3570	0.974
Toluene	6040	6240	0.969
Cyclohexane	5100	5430	0.939

Summary

(1) The physical meaning of Rao's molar sound velocity (K) is given.

(2) It is found that K-values calculated from the theory are independent of temperature in the case of normal liquids.

(3) It is found that the calculated K-values are satisfactorily coincident with the observed ones for normal liquids (aliphatic and aromatic hydrocarbons, ether, acetone, esters, alkyl halides).

12) As the sound velocity, u is ordinarily expressed in the unit of m./sec., so K_{obs} must be converted in the c. g. s. unit. Namely, ordinary K must be multiplied by $(100)^{1/3}=4.62$.

The author expresses his hearty thanks to Prof. Dr. N. Sata of the Osaka University for his valuable encouragement throughout this task. The author also expresses his sincere thanks to Mr. M. Okuyama of the Osaka University for his valuable discussions and Dr. O. Nomoto of the Kobayashi Institute of Physical Research for his kind suggestions, although his opinion is not always consistent with the author's own.

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Stoichiometric Investigations of the Liquid State. II. The Relation between the Sound Velocity and the Chemical Structure. (2). The Additivity of the Molar Sound Velocity of Unassociated Liquids

By Kiyoshi TYUZYO[1]

(Received April 17, 1954)

Introduction

According to the first paper of this series[2], Rao's molar sound velocity[3] ($K=u^{1/3}V$) is derived from the simple theory of the liquid state and the calculated molar sound velocity is independent of temperature. Furthermore, the calculated and observed molar sound velocities are coincident with each other with the accuracy of ±5%. The molar sound velocity, K, is expressed by the following equation from the theory,

$$K=\left[\frac{E_{\text{vap}}\gamma^{1/2}}{C(MRT)^{1/2}}\right]^{1/3}V \qquad (1)$$

The previous paper may be referred to about the meaning of notations in Eq. 1.

Rao[4] found experimentally that K has the additive property. The additivity of K for normal unassociated liquids will be explained theoretically in this paper.

Calculation of "Atomic-" and Radical Sound Velocity"

If K has the additive property, it is reasonable to assume as follows on the basis of Eq. 1.

$$K=\Sigma K_a \qquad (2)$$

$$K_a=\frac{\gamma^{1/6}}{C^{1/3}(RT)^{1/6}}\left[\frac{\Delta E}{M_a^{1/2}}\right]^{1/3}V_a \qquad (3)$$

In Eq. 2 and 3, M_a, V_a mean the corresponding values for each constitutive atom or radical and ΔE is the energy of vaporization per each element. This value can be approximately substituted by the cohesive energy as shown below. K_a may be named as "atomic-" or "radical sound velocity". Lagemann and others[5] have already pointed out that the additivity of K does not completely hold but some constitutive effect is present. As K_a contains the packing number and energy factor (C, ΔE), so it is natural that some constitutive effect is present as long as C and ΔE-values are related with the intermolecular interaction. Here, the author does not enter into such a detailed discussion as the influence of intermolecular interaction but calculates only K-values as the first approximation.

Because the packing number and ratio of specific heats seem to be not so remarkably dependent on the species of molecules, and, moreover, because γ enters with the 1/6 power, C enters with the 1/3 power in Eq. 3, γ and C are put equal to 1.4 and 2 respectively. In the ordinary range of liquid (0~100°C), absolute temperature (T) is nearly 300~400° and furthermore, it enters in Eq. 3 with the 1/6 power, and so T is put equal to 300 approximately. Moreover, if ΔE and R are expressed in cal/mol, then Eq. 3 becomes the following.

$$K_a=5.42\times\left[\frac{\Delta E}{M_a^{1/2}}\right]^{1/3}V_a \qquad (4)$$

where, of course, $M_a=\sum_i M_{ai}$. M_{ai} is the atomic weight that constitutes the atoms or radicals and V_a is the sum of the constituting atoms (V_{ai}) in the corresponding state.

V_a may be put equal to $\sum_i V_{ai}$ approximately[6]. This approximation is quite satisfactory for the present case. (V_{ai} is the atomic volume in the liquid state.)

ΔE corresponds to the cohesive energy and M.

1) Present address: Research Laboratory, Dai-Nippon Celluloid Co. Ltd., Sakai City, Osaka.
2) K. Tyuzyo, The first paper of this series.
3) M. R. Rao, *Indian J. Phys.*, **14**, 109 (1940).
4) M. R. Rao, *J. Chem. Phys.*, **9**, 682 (1941).
5) R. T. Lagemann, J. S. Evans, and D. R. McMillan, Jr. *J. Amer. Chem. Soc.*, **70**, 2996 (1948).
6) Even the proportionality does not hold between the so-called zero-point atomic volume in the solid state and the atomic volume in the liquid state. Therefore, the atomic volume in the liquid state must be adopted as the V_{ai}-value.

Dunkel has already found the approximate additivity for this quantity[7].

Now, we can calculate atomic or radical sound velocity (K_a) from Eq. 4. The calculated K_a-values from Eq. 4, the observed ones, and the K_{acalc}/K_{aobs} are shown in Table I. It is clear from the table that the calculated K_a-values are approximately equal to the observed ones with the accuracy of about ±10%.

If several assumptions and approximations made for the above calculation are allowed for, this result seems to be quite satisfactory.

TABLE I

CALCULATED AND OBSERVED ATOMIC OR RADICAL SOUND VELOCITY (K_a)

Atom or radical	ΔE[8] (cal/mole)	M_a	V_a[9]	K_{acalc}	K_{aobs}[10]	K_{acalc}/K_{aobs}
$-CH_2-$	990	14.0	22.0	763	905	0.850
$-CH_3$	1780	15.0	27.5	1154	1330	0.868
$-O-$	1630	16.0	7.8	313	345	0.907
$-Cl$	3400	35.5	22.1	993	1055	0.941
$-Br$	4300	79.9	27.8	1179	1137	1.037
$-I$	5040	126.7	37.5	1550	1300	1.191
$>C=O$	4270	28.6	18.8	949	860	1.103
$=CH$	1780	14.0	(22.0)[10a]	(930)	1416	(0.795)

TABLE II

THE CALCULATED AND OBSERVED MOLAR SOUND VELOCITY

Substance	Chemical Formula	$K_{obs}=u^{1/3}V$	$K_{calc}=\Sigma K_{acalc}$	K_{calc}/K_{obs}	Literature
n-Pentane	C_5H_{12}	5390	4610	0.855	(11)
n-Hexane	C_6H_{14}	6260	5380	0.859	〃
n-Heptane	C_7H_{16}	7130	6150	0.863	〃
n-Octane	C_8H_{18}	7990	6910	0.865	〃
n-Nonane	C_9H_{20}	8650	7680	0.888	〃
Ethyl bromide	C_2H_5Br	3335	3100	0.929	(12)
Ethyl iodide	C_2H_5I	3570	3470	0.972	〃
n-Propyl chloride	C_3H_7Cl	4210	3680	0.874	(5)
n-Propyl bromide	C_3H_7Br	4200	3870	0.922	〃
n-Butyl chloride	C_4H_9Cl	5070	4450	0.878	〃
n-Butyl bromide	C_4H_9Br	5020	4640	0.925	〃
n-Butyl iodide	C_4H_9I	5230	5010	0.958	(12)
Methylene chloride	CH_2Cl_2	3040	2760	0.909	(5)
Methylene bromide	CH_2Br_2	3210	3130	0.975	〃
Methylene iodide	CH_2I_2	3690	3870	1.048	(12)
Ethylene chloride	$C_2H_4Cl_2$	3910	3520	0.901	(5)
Ethylene bromide	$C_2H_4Br_2$	4010	3890	0.970	〃
Propylene chloride	$CH_2ClCH_2CH_2Cl$	4760	4290	0.901	〃
Propylene bromide	$CH_2BrCH_2CH_2Br$	4820	4160	0.863	〃
Ethyl ether	$C_2H_5OC_2H_5$	4820	4160	0.863	(2)
Acetone	CH_3COCH_3	3620	3260	0.900	〃
Methyl acetate	CH_3COOCH_3	3890	3570	0.917	(13)
Ethyl acetate	$CH_3COOC_2H_5$	4660	4330	0.929	〃

7) M. Dunkel, *Z. phys. Chem.*, A**138**, 42 (1928).
8) H. Mark and A. V. Tobolsky, "Physical Chemistry of High Polymeric Systems," p. 144 (1950).
9) S. Glasstone, "Textbook of Physical Chemistry," p. 525 (1946).
10) R. T. Lagemann, and W. S. Dunbar, *J. Phys. Chem.*, **49**, 425 (1945).
10a) As the correction for the atomic volume for the double bond is not made, the K_{acalc} of (CH=) is somewhat inaccurate.
11) L. Bergmann, "Der Ultraschall," (1949).
12) R. T. Lagemann, D. R. McMillan, Jr., and W. E. Woolf, *J. Chem. Phys.*, **17**, 369 (1949).
13) R. T. Lagemann, E. G. McLeroy, and O. Milner, *J. Amer. Chem. Soc.*, **73**, 5891 (1951).

Comparison of the Observed Molar Sound Velocities and the Calculated Ones from the Atomic and Radical Sound Velocities.

As an application of the above result, the calculated K from K_{acalc}-values in Table I and the observed ones for unassociated liquids are shown in Table II. The consistency of K_{calc} and K_{obs} is quite satisfactory, and so the above method of calculation is fully reasonable as the first approximation.

Discussion

If the quantities which may be regarded as constant in Eq. 1 are written A, then

$$K=A\left(\frac{E_{vap}}{M^{1/2}}\right)^{1/3}V \qquad (5)$$

From the assumption of Eq.2 and 3, Eq. 5 is

$$K=\Sigma K_a=A\Sigma\left[\left(\frac{\Delta E}{M_a^{1/2}}\right)^{1/3}V_a\right] \qquad (6)$$

On the other hand, from the assumptions,

$$\left.\begin{aligned} M&=\Sigma M_a,\\ E_{vap}&=\Sigma \Delta E,\\ V&=\Sigma V_a. \end{aligned}\right\} \qquad (7)$$

Therefore, from Eq. 5 and 7,

$$K=A\left[\frac{\Sigma(\Delta E)}{(\Sigma M_a)^{1/2}}\right]^{1/3}(\Sigma V_a) \qquad (8)$$

In general, Eq. 6 is not consistent with Eq. 8, and Eq. 8 contains many more cross terms than Eq. 5. Therefore, it is not clear at present whether the discrepancy (about 10%) between the calculated and observed values in Table II is due to the existence of these cross terms or the constitutional effect of ΔE and V coming from the intermolecular interaction. But, considering the fact that the calculated and observed K_a (Table I) has already about 10% difference, the influence of the constitutive effect of ΔE and V seems to be larger than that of the cross terms. (If the influence of cross terms is large, the deviation of the calculated K-value must be larger than that of the calculated K_a-value.) Of course, more detailed investigation is necessary for this point.

Next, as a useful application of Eq. 4, molar cohesion, ΔE, can be reversely calculated if K_a, M_a, V_a are known. ΔE, is derived from the temperature-variance of vapor pressure until now and that method is considerably difficult, but from Eq. 4, its value is very simply calculated.

Summary

(1) The additivity of molar sound velocity has theoretically been derived and the calculated and observed "atomic-" and "radical sound velocity" were shown to coincide with each other with the accuracy of about ±10%.

(2) The molar sound velocity derived from the calculated atomic and radical sound velocity was shown to coincide with the observed value with about 10 % deviation for unassociated liquids. (e. g., paraffins, alkyl halides, ethers, ketones, esters)

(3) The cause of deviation of the calculated and observed values and the application of this theory are briefly discussed.

The author expresses his hearty thanks to Prof. Dr. N. Sata and Mr. M. Okuyama of the Osaka University for their encouragement in this work. The author also expresses his sincere thanks to Dr. O. Nomoto of the Kobayashi Institute of Physical Research for his kind suggestions, although his opinion is not always consistent with the author's own.

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Stoichiometric Investigations of the Liquid State. III. The Relation between the Sound Velocity and the Chemical Structure. (3). Molar Sound Velocity and its Additivity for Associated Liquids

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Introduction

The author theoretically calculated the molar sound velocity of unassociated liquid and found its calculated value to be independent of temperature and to be satisfactorily consistent with the observed value[2]. Then, the author interpreted the additivity of the molar sound velocity and also showed that the calculated atomic or radical sound velocity was satisfactorily consistent with the observed one[3]. In this paper, results of the same analysis for associated liquids will be reported.

The Temperature Variation of the Molar Sound Velocity of Associated Liquids

According to the first paper, Rao's molar sound velocity, R, is expressed by the following equation.

$$R=\left[\frac{E_{\mathrm{vap}}\tau^{1/2}}{c(MRT)^{1/2}}\right]^{1/2}V \qquad (1)$$

The previous paper may be referred to about the meaning of notations in Eq. 1.

The temperature variation of R calculated from Eq. 1 is shown in Table I. There are few substances of which all values in Eq. 1 are complete. They are only water, methyl alcohol and ethyl alcohol. As the special case, the liquefied gas, oxygen and nitrogen, are also added in the table.

TABLE I

TEMPERATURE VARIANCE OF MOLAR SOUND VELOCITY OF ASSOCIATED LIQUID AND LIQUEFIED GAS

Temperature (°C)	E_{vap} (cal/mole)	ρ (g/cc)	R_{calc}
Water (M=18.0, τ=1.336)			
0	10170	1.000	1310
10	10050	1.000	1300
20	9920	0.998	1270
30	9800	0.996	1275
40	9700	0.992	1270
50	9570	0.988	1260
60	9460	0.983	1260
70	9340	0.978	1255
80	9210	0.972	1250
Methyl alcohol (M=32.04, τ=1.203)			
0	8540	0.810	2420
10	8450	0.801	2430
20	8350	0.791	2430
30	8220	0.782	2410
40	8080	0.772	2430
50	7950	0.763	2440
Ethyl alcohol (M=46.07 τ=1.13)			
0	9550	0.806	3380
10	9480	0.798	3390
20	9400	0.789	3395
30	9320	0.781	3405
40	9250	0.772	3410
50	9130	0.764	3420
Oxygen (M=32.00, τ=1.425)			
−210	306	1.272	978
−200	270.5	1.224	955
−190	234	1.176	926
−185	217	1.152	909
Nitrogen (M=28.02, τ=1.42)			
−203	192	0.843	1110
−201	184.5	0.834	1110
−199	177	0.824	1100
−197	170	0.815	1090

It is clear from the table that R of water, liquid oxygen and liquid nitrogen are considerably dependent on temperature, but R of alcohols are nearly independent of temperature like unassociated liquids. Weissler accurately measured the sound velocity in a number of alcohols and calculated the molar sound velocity on the bases of the definition, $R=u^{1/3}V$.[4] Then, he found that R of alcohols are independent of temperature, in contrast with water. This result is quite consistent with the above result of calculation.

In the calculation from Eq. 1, all values are observed ones except C=2. But whether C=2 or not is questionable. On trial, C was calculated from sound velocity by the following equation[2].

$$C=\frac{E_{\mathrm{vap}}\tau^{1/2}}{u(MRT)^{1/2}} \qquad (2)$$

1) Present Address: Research Laboratory, Dai-Nippon Celluloid Co. Ltd., Sakai City, Osaka.
2) K. Tyuzyo, This Bulletin, **27**, 300 (1954).
3) K. Tyuzyo, This Bulletin, **27**, 303 (1954).
4) A. Weissler, *J. Amer. Chem. Soc.*, **70**, 1634 (1948).

The calculated temperature variation of C is shown in Table II. It is clearer than in Table I that C of water, liquid oxygen, and liquid nitrogen are dependent on temperature but those of alcohols are almost constant.

Table II

Temperature variance of packing number, C

temperature (°C)	C
water	
0	5.47
10	5.15
20	4.69
30	4.67
40	4.48
50	4.30
60	4.17
70	4.05
80	3.94
liquid oxygen	
−210	1.04
−200	0.93
−190	0.81
−185	0.76
liquid nitrogen	
−203	0.81
−201	0.79
−199	0.76
−197	0.73
methyl alcohol	
0	3.87
10	3.86
20	3.86
30	3.86
40	3.84
50	3.83
ethyl alcohol	
0	3.34
10	3.36
20	3.38
30	3.40
40	3.41
50	3.42

In Table III C-values at 20°C are shown for several associated liquids. The C-values in the third row are the calculated ones from vapor pressure by Eyring and others[5]. The values of C of water, ethylene glycol, and glycerol are much larger than 2, while on the contrary, C those of liquid oxygen and nitrogen, are much smaller than 2. However, in methyl and ethyl alcohol the value is much larger than 2, and the higher the alcohol, the smaller C becomes and its value converges to 2. For fatty acids C is generally 2 except formic and acetic acid. The above facts will be discussed later. Perhaps these are due to the difference in the associated state of the associated liquid.

4) H. Eyring and J. Hirshfelder, *J. Phys. Chem.*, **41**, 249 (1937).

Table III

Packing number, C, of associated liquids (20°C)

Substance	C	C from vapor pressure
water	4.69	—
ethylene glycol	3.17	—
glycerol	2.74	—
oxygen	0.93 (−200°C)	—
nitrogen	0.79 (−201°C)	—
mercury	—	0.21
methyl alcohol	3.86	0.43
ethyl alcohol	3.38	—
n-propyl alcohol	2.57	—
n-butyl alcohol	2.36	—
n-amyl alcohol	2.26	—
n-hexyl alcohol	2.16	—
n-heptyl alcohol	2.10	—
n-octyl alcohol	2.03	—
formic acid	1.46	—
acetic acid	1.51	—
n-propionic acid	1.72	—
n-butylic acid	2.17	—
n-valeric acid	2.04	—
n-caproic acid	1.94	—
n-heptylic acid	1.86	—
n-caprylic acid	1.82	—

The Calculated Values of Molar Sound Velocity

Table IV shows the calculated (C is put equal to 2) and observed molar sound velocity, K. K_{calc} of water, ethylene glycol, glycerol, oxygen, nitrogen, methyl alcohol, ethyl alcohol, formic acid, and acetic acid are considerably different from K_{obs}, but others are consistent with K_{calc}. (about 5% deviation).

Table IV

Calculated and observed values of molar sound velocity, K

Substance	K_{calc}	K_{obs}	K_{calc}/K_{obs}
water	1070	955	1.198
ethylene glycol	3520	3020	1.167
glycerol	4700	4220	1.114
oxygen	955	1234	0.774
nitrogen	1110	1490	0.747
methyl alcohol	2430	1955	1.242
ethyl alcohol	3400	2860	1.188
n-propyl alcohol	3920	3720	1.053
n-butyl alcohol	4620	4625	0.999

n-amyl alcohol	5300	5440	0.973
n-hexyl alcohol	6120	6380	0.958
n-heptyl alcohol	6950	7200	0.965
n-octyl alcohol	7630	8080	0.944
formic acid	1630	1905	0.857
acetic acid	2360	2780	0.850
n-propionic acid	3240	3650	0.888
n-butylic acid	4410	4530	0.974
n-valeric acid	5110	5430	0.942
n-caproic acid	5830	6300	0.927
n-heptylic acid	6550	7210	0.908
n-caprylic acid	7260	8090	0.899

Additivity of Molar Sound Velocity

According to the second paper of this series[3], the molar sound velocity, K, is expressed as the sum of atomic or radical sound velocities, K_a. Namely,

$$K = \sum K_a \tag{3}$$

$$K_a = 5.42 \times \left[\frac{\Delta E}{M_a^{1/2}} \right]^{1/3} V_a \tag{4}$$

The previous paper may be referred to about the meaning of notations in Eq. 4.

Table V shows the radical sound velocities of hydrogen-bonding radicals calculated from Eq. 4. Generally, the calculated value is larger than the observed, but the deviation is not much larger than that of the normal radicals except amine-radical.

Table VI shows the calculated K using the valve of Table V and the observed one of associated liquids. The consistency is generally satisfactory. Perhaps, the fact that the deviations of $-CH_2-$ and $-OH$ radical compensate each other is the cause of this fact.

TABLE V

RADICAL SOUND VELOCITY OF HYDROGEN-BONDING RADICALS

radical	ΔE(cal/mole)[6]	M_a	V_a[7]	K_{acalc}	K_{aobs}	K_{acalc}/K_{aobs}
—OH	7250	17.0	13.3	869	773	1.124
—COOH	8970	45.0	32.1	1910	1633	1.170
—SH	4250	33.1	28.1	1308	—	—
$-NH_2$	3530	16.0	21.5	1116	842	1.325

TABLE VI

CALCULATED K AS THE SUM OF ATOMIC OR RADICAL SOUND VELOCITY AND OBSERVED ONES FOR ASSOCIATED LIQUIDS

substance	K_{obs}	K_{calc}	K_{calc}/K_{obs}	literature of K_{obs}
water	955	—	—	(8)
ethylene glycol	3020	3272	1.083	〃
glycerol	4220	—	—	〃
methyl alcohol	1955	2023	1.033	(4)
ethyl alcohol	2860	2790	1.011	〃
n-propyl alcohol	3720	3557	0.956	〃
n-butyl alcohol	4625	4324	0.935	〃
n-amyl alcohol	5440	5091	0.937	〃
formic acid	1905	—	—	(8)
acetic acid	2780	3064	1.101	〃
n-propionic acid	3650	3831	1.049	〃
n-butylic acid	4530	4600	1.014	〃
n-valeric acid	5430	5365	0.988	〃

Discussion

Summarizing the results of the previous sections.

(1) The calculated K of alcohols are almost independent of temperature, but that of water is remarkably dependent on temperature. This fact is clearer from the packing number, C. These results are censistent with the experimental results of Weissler. Below, for convenience, the associated liquids such as alcohols and fatty acids series are named as "the associated liquids of the first kind" and water, ethylene glycole, glycerol, etc. are named as" the associated liquids of the second kind."

(2) Packing number is much larger than 2 (normal value) for the associated liquids of the second kind, but is almost 2 for the asoociated liquids of the first kind except

6) H. Mark and A. V. Tobolsky, "Physical Chemistry of High Polymeric Systems," p. 144 (1950).
7) S. Glasstone, "Textbook of Physical Chemistry," p. 525 (1946).
8) L. Bergmann, "Der Ultraschall," p. 263 (1949).

the initial two members of the homologous series.

(3) The consistency of the calculated and observed K is unsatisfactory for the associated liquids of the second kind, but is satisfactory in those of the first kind except the initial two members of the homologous series.

(4) The calculated radical sound velocity is consistent with the observed value with the deviation of about 15% and the calculated molar sound velocity based on the additivity principle is satisfactorily consistent with the observed value in the associated liquids of the first kind. (Data for the associated liquids of the second kind are lacking, but the calculated value of K of ethylene glycol is consistent with the observed one).

Until now, methods of inferring the state of association of associated liquids from sound velocity were those of Lagemann[9] and van Itterbeek[10], but these are both unsatisfactory even qualitatively, as well as quantitatively. The results obtained in this paper are also not so striking, but it seems to be reasonable to divide the associated liquids into the first and the second kind. The author already showed that the associated liquids must be distinguished in the first and second kind by the analysis of the viscous flow[11]. It is a very interesting fact that this is also reasonable in the case of molar sound velocity. According to the author's opinion, it is probable that in the associated liquids of the second kind, there exist no clearly distinguishable associated units and they have a network structure as a whole, and their structures are remarkably deformable and also destroyed with the increase of temperature. In this case it is natural that molar sound velocity shows the several abnormalities as are shown above. On the other hand, in the associated liquids of the first kind, there exist clearly distinguishable associated units, and hydrogen bond connecting this unit is almost strong enough not to be influenced by temperature. Then, except the fact that the unit is large, the molecules of the associated liquids of the first kind are the same as the molecules of the normal liquids. Moreover, $E_{vap.}$, V of Eq. 1 and ΔE, V_a of Eq. 4 contain already the factors of the associated state and so molar sound velocity has no abnormalities. Abnormalities of formic acid and acetic acid probably depend on the special type of association in the liquid state[11,12]. Abnormalities of methyl and ethyl alcohol can not be explained at present.

Lastly, among the methods of determining the state of association of the associated liquids, those methods are quite unsatisfactory which contain the energy of vaporization as an important factor; for example, surface tension, parachor[13] and sound velocity. In the energy of vaporization of the associated liquids, dissociation energy of associated units is contained in addition to the normal cohesive energy. Therefore, it seems to be very difficult to obtain any knowledge about the state of association from the vaporization phenomenon[14]. Those methods which use the properties of the liquids themselves such as viscosity are more effective than those which depend on the quantity of the equilibrium state of liquids and gases such as energy of vaporization for studying the state of association[11,12].

Summary

(1) Associated liquids are analysed with a method similar to that of the previous papers, and the temperature variation of molar sound velocity, comparison with experiment, calculation of the radical sound velocity, additivity, etc. are developed.

(2) It seems to be reasonable to distinguish the associated liquids to the first and second kind. This is consistent with the results of the author's investigation of the viscous flow.

(3) Something about the structure of the associated liquids of the first and second kind, and methods of investigating the state of association are discussed.

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9) R. T. Lagemann, *J. Chem. Phys.*, **12**, 464 (1948).
10) A. van Itterbeek and A. De Bock, *Nature*, **162**, 611 (1948).
11) K. Tyuzyo, *Kolloid-Z.*, **131**, 40 (1953).
12) K. Tyuzyo, The fourth and fifth paper of this series, to be published elsewhere.
13) S. Sugden, "Parachor and Valency", Chap. VIII(1930).
14) K. Tyuzyo, The sixth paper of this series, to be published elsewhere.

LETTER

Die Einwirkung der Wechselspannung auf die Tropfzeit der Quecksilbertropfelektrode

Von Isamu TACHI und Mitsuko OKUDA

(Eingegangen am 27. Feb. 1954)

Die Tropfzeit der in die elektrolytische Lösung getauchten Quecksilbertropfelektrode, whelche sich als eine vollkommen polarisierte Elektrode varhält, verändert sich bekanntlich mit der Variation der Gleichstromspannung, die zwischen der Tropfelektrode und der unpolarisierbaren Gegenelektrode angelegt ist, und zwar beobachtet man eine annähernd parabolische Tropfzeit(τ)-Spannung(E)-Kurve, deren Maximum bei -0.4—0.7 Volt vs. norm. Kalomelelektrode liegt. Die Tropfzeit ist nach der Tateschen Theorie ergeben durch

$$\tau = \frac{2\pi r}{m} \cdot \sigma,$$

worin τ = Tropfzeit in Sek., r = Innerer Radius der Kapillare in cm., m = Ausflussgeschwindigkeit des Quecksilbers in g/Sek., und σ = Oberflächenspannung der Quecksilbertropf in dyn./cm. Hierin ist r/m bei der angegebenen Kapillare und Niveauhöhe konstant. Daher zeigt die τ-E-Kurve die Abhängigkeit der Hg-Lösung-Oberflächenspannung; d. h. es besitzt dieselbe Eigenschaft wie die Elektrokapillarkurve.

Neuerlich haben Frau Fournier[1] und Breyer mit seinem Kollegen[2] die Wechselspannungpolarographie untersucht, worin eine Wechselspannung von geringer Amplitude der gewöhnlichen Gleichstromspannung überlagert wird. Wir haben auch diese neue Art der polarographischen Elektrolyse versucht, und eine neue Erscheinung von der Diskontinuität in τ-E-Kurve gefunden. Also haben wir die Einwirkung der Wechselspannung auf die Tropfzeit versucht. Die verwandte Lösung war 0.1 N KCl, worin das Wasserstoffgas acht Stunden durchgeleitet worden war, und die für zehn Tropfen erforderliche Zeitdauer wurde gemessen. Das Schaltbild ist in Abb. 1 angegeben, und in Abb. 2 ist die Tropfzeit gegen die Spannung aufgetragen. Die Überlagerung der Wechselspannung veranlässt keine beträchtliche Veränderung

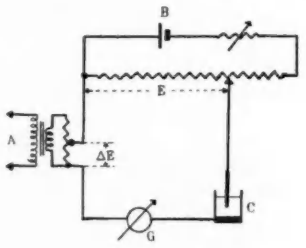

Abb. 1.
Schaltbild
A: Sinusförmige Wechselspannung von 60 Hz.
B: 2∼4 Volt. Akkumulator
C: Quecksilbertropfelektrode
G: Galvanometer

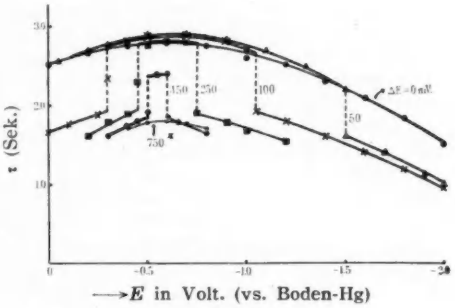

Abb. 2.
τ-E-Kurve
(0.1 norm. KCl bei gewöhnlicher Temperatur)
* Wenn die Amplitude der überlagerten Wechselspannung 755 mV. übersteigt, so wird die Tropfzeit irregulär.

der τ-E-Kurve in der Nähe des dem Elektrokapillarmaximum entsprechenden Potentials. Jedoch, wenn die angelegte Spannung negativer wird und einen gewissen kritischen Potentialwert übersteigt, so ist eine diskontinuierliche Verminderung der Tropfzeit bemerkbar. Infolgedessen stossen wir die Schwierigkeit in der polarographischen Messung der Wellenhöhe auf. Dieselbe Erscheinung ist auch in Bereich der positiveren Gleichstromspannung erkennbar, und der ganze Verlauf der τ-E-Kurve ist in Abb. 2 angegeben. Die beiden kritischen Potentialwerten sind von der Amplitude der überlagerten Wechselspannung abhängig. Abb. 2 zeigt auch den Einfluss der Amplitude (ΔE: Effektivwert) der überlagerten Wechselspan-

1) M. Fournier; *Comp. rend.* **237**, 1673 (1951).
2) B. Breyer und F. Gutman; *Discuss. Faraday Soc.* **1**, 19 (1647).

nung auf die τ-E-Kurve. Daraus ist zu ersehen, dass sich die beiden kritischen Potentialwerten an der kathodischen und anodischen Asten mit wachsender Amplitude etwa symmetrisch zum Elektrokapillarmaximum annähern (siehe Abb. 3); und zwar nach der plötzlichen Veränderung der Tropfzeit, verläuft die τ-E-Kurve zur eigentlichen Elektrokapillarkurve annähernd parallel.

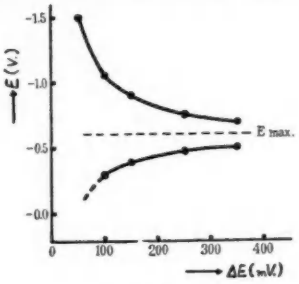

Abb. 3.
Schematische Darstellung des Zusammenhanges zwischen den beiden kritischen Potentialwerten und der überlagerten Wechselspannungsamplitude

Worauf beruht diese Erscheinung, ist theoretisch nicht aufgeklärt; jedoch wäre es zur Untersuchung der Elektrokapillarität bzw. der elektrischen Doppelschicht von Bedeutung.

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